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Comparative Toxicity of Eight Model Substances to the Sediment Dwelling Invertebrates *Lumbriculus variegatus* and *Chironomus riparius*

vorgelegt von
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Abbreviations

EC_X	$\widehat{=}$	A statistically derived concentration that causes x% sublethal effect of the test organisms over a defined period of exposure
LC_X	$\widehat{=}$	A statistically derived concentration that causes x% mortality of the test organisms over a defined period of exposure
LOEC	$\widehat{=}$	Lowest observed effect concentration
NOEC	$\widehat{=}$	No observed effect concentration
log K_{ow}	$\widehat{=}$	n-octanol-water partition coefficient
C.r.	$\widehat{=}$	*Chironomus riparius*
L.v.	$\widehat{=}$	*Lumbriculus variegatus*
t0	$\widehat{=}$	time at the start of the exposure period
t48h	$\widehat{=}$	time at the end of the exposure period after 48 hours
t96h	$\widehat{=}$	time at the end of the exposure period after 96 hours
t28	$\widehat{=}$	time at the end of the exposure period after 28 days

1 Introduction

The term ecotoxicology was first introduced by the toxicologist Truhaut in 1969 to combine the fields of ecology and toxicology. Ecotoxicology studies the toxic effects of environmental contaminants to the constituents of ecosystems (TRUHAUT, 1977). Historically, the evaluation of contaminants has focused on water exposition. The primary incentive for sediment toxicity testing has been dredge material permitting in the United States of America in the late 1970s (INGERSOLL, 1995). In the aquatic environment, most anthropogenic chemicals and waste materials, including toxic organic and inorganic chemicals, eventually accumulate in sediment (INGERSOLL, 1995). Discharged contaminants to surface waters adsorb to particulate matter and accumulate in sediments and may thus be a burden to benthic organisms. Concentrations of chemicals can be multiple higher in sediments than in surface water. Since the sediment is a habitat for many benthic organisms, these organisms can be exposed to these pollutants to an extremely high degree. Sediment-dwelling organisms can be exposed to contaminants that have accumulated in sediments either by direct contact via remobilized contaminant into the water phase or by ingestion of contaminated sediment. Accumulation in the organism via the water phase is defined as bioconcentration; accumulation via food is described as biomagnification. Sediment-dwelling organisms are a major food source for higher trophic levels in the food chain. Because benthic invertebrates readily accumulate chemicals with high accumulation potential via ingestion of food and interstitial water concentrations from sediments (REICHERT *et al.*, 1985; LANDRUM, 1989; LANDRUM *et al.*, 1991; CLEMENTS *et al.*, 1994), dietary uptake by benthic-feeding fish may be a significant route of exposure (CLEMENTS *et al.*, 1994) in addition to incidental ingestion of contaminated sediment (NIIMI & DOOKHRAN, 1989). Thus, contaminants may reach higher trophic levels by bioaccumulation in the food chain.

The invertebrates *Chironomus riparius* and *Lumbriculus variegatus* were selected for sediment toxicity testing as representatives of endobenthic living organisms. *C. riparius* has commonly been used for testing of contaminated sediments as well as in studies that spike artificial sediment with contaminants of potential sediment accumulation (WENTSEL *et al.*, 1978; INGERSOLL *et al.*, 1995; DAY *et al.*, 1998; BERVOETS *et al.*, 2004; LOTUFO & FARRAR, 2005). *C. riparius* spend the main part of their life cycle as larval stages in the sediment feeding on organic matter. *L. variegatus* ingests sediment particles while burrowing in the sediment. Both organisms are living in the sediment and are therefore ideal for assessing the toxic effects of sediment-associated contaminants.

Existing ecotoxicity data for chemicals vary to a high extent between the environmen-

tal compartments water and sediment, since the evaluation of contaminants has historically focused on water exposition. Toxicity data for algae, daphnids, and fish (via water-only exposure) are available for many substances, since they belong to the so called base set. The existing sediment toxicity data are rather rare. Thus, the interest arises to predict sediment toxicity for sediment-dwelling invertebrates from existing acute toxicity data of tests with water-only exposure. The main emphasis of this work was placed on one metal compound (cadmiumchloride) and seven organic chemicals that persist and bioaccumulate. 4,4-dichlorodiphenyltrichloroethan (DDT), benzo-[a]-pyrene (B(a)P), pentachlorophenol (PCP), 2,4-dichlorophenol (2,4-DCP), and trinitrotoluene (TNT) were selected to cover a relatively wide range of lipophilicity. 3,4-dichloroaniline (3,4-DCA) was selected for its covalent binding characteristics to organic matter. Tributyltin was chosen both as an organometallic compound and because of its high environmental relevance. Sediments spiked with known concentrations of contaminants can be used to determine concentration effect relationships between chemicals and biological effects on the selected organism.

The objective of this study was fivefold: (1) develop methods and improve existing procedures on acute and sediment toxicity testing of the two invertebrates; (2) conduct both acute toxicity tests via water exposure and long-term sediment toxicity tests for the selected model substances to generate data for comparative discussion; (3) assess correlations among acute toxicity data of the organisms exposed via water-only and correlations among sediment toxicity data of the two endobenthic invertebrates for the eight tested chemicals; (4) assess possible forecasting for sediment toxicity from acute toxicity (via water-only exposure) and (5) assess exposure effects to determine the main exposure route.

Further, the usage of one sediment with the same sediment composition and the same water-to-sediment ratios for both invertebrates was discussed, to have similar exposure conditions.

This study is part of a larger study that aimed to determine the toxicity of selected substances to organisms inhabiting sediment and organisms inhabiting the terrestrial compartment, and to determine possible relationships among aquatic, sediment, and terrestrial toxicity data.

2 Methods

2.1 Biology of the test organisms

2.1.1 *Lumbriculus variegatus*

L. variegatus (Annelida; Clitellata; Oligocheata; Lumbriculidae) is found throughout North America and Europe. In nature, *L. variegatus* (see figure 2.1) can reproduce sexually. After

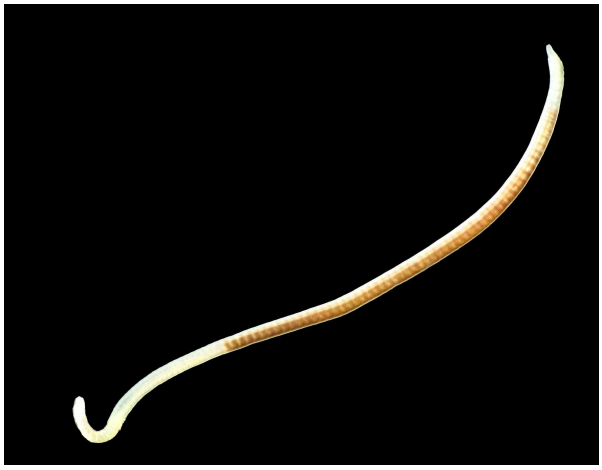


Figure 2.1: *L. variegatus* (printed with permission from EGELER *et al.* (2005))

copulation and sperm exchange (the latter was never documented), worms produce transparent cocoons, each containing 4 to 11 fertilized eggs that undergo direct embryonic development with no larval stage (DREWES & BRINKHURST, 1990). Reproduction under laboratory conditions is always by fragmentation (morphallaxis). Fragments can develop into complete individuals by regenerating a new head, a new tail, or both (DREWES & BRINKHURST, 1990). *L. variegatus* was obtained from BIO - International (Netherlands) in 1994. The animals were kept in continuously aerated tap water in 10 l glass aquaria at a temperature of 20 °C. Quartz sand of a grain size of 62 μm to 2000 μm was used as sediment. Animals were fed once a week with TetraMin® *ad libitum*. The water was changed every 2 weeks and sediment was thoroughly washed every 2 month.

2.1.2 *Chironomus riparius*

Chironomids (see figures 2.2 and 2.3) are widely distributed and are frequently the most abundant insects in freshwater. The imagines of the non-biting midge *C. riparius* (Arthropoda; Insecta; Diptera; Chironomidae) usually breed within 24 hours after emergence. Females extrude gelatinous egg clutches into the water. Larvae hatch after 2 to 4 days. Larvae undergo four developmental stages before pupation. Adults emerge within 13 to 25 days at 20 °C. Lar-

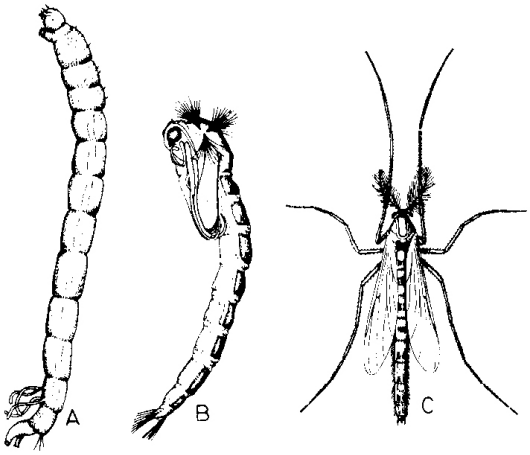


Figure 2.2: *Chironomus* sp., A larvae ca. 20 mm, B pupae 11 mm, C adult (male) 7 mm (ENGELHARDT, 1955)

(a) male imago (b) female imago

Figure 2.3: *C. riparius*

vae feed as collector-gatherers on deposited material and submerged substrate (RASMUSSEN, 1984). As tube-dwelling organisms they feed by extending the anterior part of the body outside the tube while using the posterior pro-legs to maintain contact with the inner surface of

the tube (RASMUSSEN, 1984). The brood stock, received from Bayer AG Leverkusen in November 1999, was cultured at 20 °C. Larvae were kept in several 5 l glass aquaria containing an artificial quartz sand layer (grain size of 62 to 2000 μm) of 1 cm thickness and dechlorinated active carbon filtered tap water. The water was renewed every week and larvae were fed three times a week with TetraMin® *ad libitum*. Sediment was thoroughly washed every 2 months.

2.2 Test substances

Trinitrotoluene, 2,4-dichlorophenol, pentachlorophenol, benzo-[a]-pyrene and 4,4-dichlorodiphenyltrichloroethan were selected to cover a relatively wide range of lipophilicity starting from a log K_{ow} of 1.6 for trinitrotoluene to 6.91 for 4,4-dichlorodiphenyltrichloroethan. Trinitrotoluene was selected because of its importance as a soil contaminant. Further, 3,4-dichloroaniline was selected. Accumulation of 3,4-dichloroaniline in sediment and soils cannot be explained solely by its relatively low log K_{ow} rather than by covalent binding to organic matter (HEIM *et al.*, 1994, 1995). Tributyltinchloride was chosen because of its importance as an organometallic compound with potent endocrine-disrupting properties in both invertebrates and vertebrates. Cadmiumchloride was selected as a metal compound. All the substances were selected for their environmental relevance and for their large database of aquatic toxicity data for algae, daphnids and fish. A wide range of aquatic toxicity data for the selected chemicals was necessary for extrapolation and correlation.

2.2.1 Cadmiumchloride

The heavy metal cadmiumchloride ($CdCl_2$, CAS No. 10108-64-2) is a naturally occurring element. Cadmium is the major part in zinc ore. Cadmium minerals are rather rare. The extraction of the cadmium takes place mainly as a by-product during zinc ore production. Cadmiumchloride has a log K_{ow} of -1.65, a water solubility of 1.4 $\mathrm{kg\,l^{-1}}$ at 20 °C, and a molar mass of 183.32 $\mathrm{g\,mol^{-1}}$. The chemical structure is shown in figure 2.4. Cadmium inhibits the Na,K-ATPase activity (KINNE-SAFFRAN *et al.*, 1993). Metals toxicity is due to several mechanisms including ionoregulatory disturbance, respiratory disturbance, and cellular damage (see references in BARRON *et al.*, 2002). Toxic cell injury is thought to be caused by unbound cadmium or free cadmium ion. Metallothionein binds cadmium and prevents the free cadmium ions from exerting their toxic effects. Toxic effects may occur when binding capacity of metallothionein is exceeded.

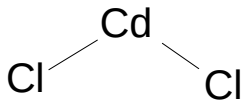


Figure 2.4: Chemical structure of cadmiumchloride ($CdCl_2$)

2.2.2 2,4,6-Trinitrotoluene

2,4,6-trinitrotoluene (TNT, CAS No. 118-96-7) is the most used explosive today. The basic material for the production of TNT is toluene. TNT is the end product of several nitrating stages. The industrial production of TNT led to a substantial load of TNT and its degradation by-products in soil and groundwater at many locations of the armaments industry (RIPPEN, 1996). TNT has a log K_{ow} of 1.6, a water solubility of 0.13 $g\,l^{-1}$ at 20 °C, and a molar mass of 227.13 $g\,mol^{-1}$. The chemical structure is shown in figure 2.5. Dinitroaromatic compounds, such as 4-amino-2,6-dinitrotoluene (4ADNT), the predominant derivative of TNT, have generally been associated with an intoxication syndrome that is consistent with a chemical reactivity-based mode of action. The toxicity of TNT and its derivatives is exerted through its enzymatic redox cycling with the formation of reactive oxygen species (DENEER *et al.*, 1987; KONG *et al.*, 1989; MASON, 1990; KUMAGAI *et al.*, 2004), or covalent binding of its reduction products to proteins and DNA (LEUNG *et al.*, 1995; HOMMA-TAKEDA *et al.*, 2002). The respiratory uncoupling mode of action is discussed by GREEN *et al.* (1999). The respiratory uncoupling mode of action is found for nitrobenzene, which is closely related to TNT and its metabolites (MANAHAN, 1992, cited by GREEN *et al.*, 1999).

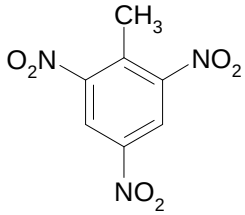


Figure 2.5: Chemical structure of 2,4,6-trinitrotoluene ($C_7H_5N_3O_6$)

2.2.3 3,4-Dichloroaniline

3,4-dichloroaniline (3,4-DCA, CAS No. 95-76-1), an aromatic amino compound, binds to the sediment by physisorption and by chemisorption. A covalent binding to organic matter was reported (HEIM *et al.*, 1994, 1995). Phosgenation of 3,4-DCA yields 3,4-dichlorophenylisocyanate (BUA, 1994). The chemical structure is shown in figure 2.6. In Germany, 3,4-DCA is used almost exclusively in the synthesis of herbicides Linuron®, Propanil®, and Diuron®. Only the herbicide Diuron® is registered for use in Germany (BVL, 2005). The treatment of

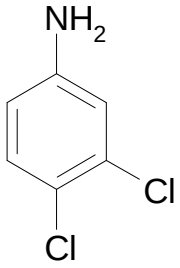


Figure 2.6: Chemical structure of 3,4-dichloroaniline ($C_6H_5Cl_2N$)

agricultural surfaces with crop protection products containing active ingredients synthesized on the basis of dichloroaniline leads to the introduction of 3,4-DCA into soil due to metabolism of the herbicides. It is possible that 3,4-DCA reaches aquatic ecosystems via run-off after application of the named herbicides on sealed surfaces. Further introduction of 3,4-DCA into the hydrosphere occurs by production and processing (BUA, 1994). 3,4-DCA has a log K_{ow} of 2.79, a water solubility of 0.6 $g l^{-1}$ at 20 °C, and a molar mass of 162.02 $g mol^{-1}$ (BUA, 1994). Dichloroanilines are methemoglobin-forming agents. The methemoglobin-forming effect is due to hydroxylated compounds formed as intermediates (see references in BUA, 1994).

2.2.4 2,4-Dichlorophenol

2,4-Dichlorophenol (2,4-DCP) is used as an intermediate for the production of phenoxyherbicides and is produced as metabolite during the biological breakdown of the herbicides. 2,4-DCP with the log K_{ow} of 2.8 has a water solubility of 4.5 $g l^{-1}$ (20 °C). The chemical structure is shown in figure 2.7. 2,4-dichlorophenol is considered to be polar narcotic (MCCARTY *et al.*, 1993). Polar narcotics elicit initial excitatory responses followed by narcotic-like depression (BRADBURY *et al.*, 1989). Narcotic chemicals cause hypoactivity and have rapidly reversible anesthetic effects. Their mode of action is a nonspecific and reversible interaction with cellular lipids and proteins (VAN WEZEL & OPPERHUIZEN, 1995).

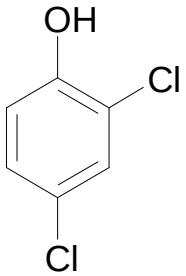


Figure 2.7: Chemical structure of 2,4-dichlorophenol ($C_6H_4Cl_2O$)

2.2.5 Pentachlorophenol

Pentachlorophenol (PCP) has a log K_{ow} of 4.74. PCP is almost insoluble at pH of 5 (0.014 gl^{-1}). Solubility increases to 3 gl^{-1} at pH 7 and 15 gl^{-1} at pH 10 (BUA, 1985). The chemical structure is shown in figure 2.8. PCP belongs to the excitatory agents, a smaller group of substituted phenolic chemicals that cause hyperactivity and overreaction to outside stimuli (MCCARTY *et al.*, 1993; PENTTINEN & KUKKONEN, 1998; BARRON *et al.*, 2002). PCP acts as an uncoupler of oxidative phosphorylation and it inhibits the synthesis of ATP thus distorting organism's energy metabolism (TERADA, 1990). PCP has widely been used as pesticide and wood preservative. Production and usage is forbidden in the European Union since 1989.

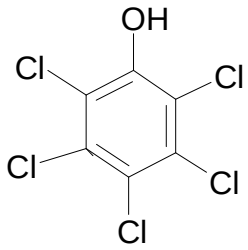


Figure 2.8: Chemical structure of pentachlorophenol (C_6Cl_5OH)

2.2.6 Benzo-[a]-pyrene

Benzo-[a]-pyrene (B(a)P) has a log K_{ow} of 6.11. B(a)P is almost insoluble at 25 °C and belongs to the family of polycyclic aromatic hydrocarbons (PAHs). The chemical structure is shown in figure 2.9. All PAHs have a so-called bay-region, a typical characteristic of carcino-

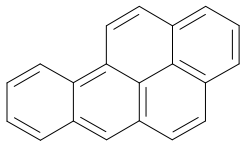


Figure 2.9: Chemical structure of benzo-[a]-pyrene ($C_{20}H_{12}$)

genic substances. Carcinogenic effects are documented for vertebrates. Biotransformation and metabolic activation of the carcinogenic B(a)P occurs primarily through the aryl hydrocarbon receptor-mediated induction of the CYP1 family of P450 monooxygenases. Further, B(a)P induces reproductive toxicity in fish (THOMAS, 1990; HOFFMANN & ORIS, 2006). One of the underlying mechanisms may be an altered transcription of genes important in regulating reproduction (HOFFMANN & ORIS, 2006). Also, PATEL *et al.* (2006) suggest that the cytochrome P450 aromatase (CYP19), which is the key steroidogenic enzyme responsible for

conversion of androgens to estrogens, is a potential target for disruption of fish developmental and reproductive physiology by B(a)P.

2.2.7 4,4-Dichlorodiphenyltrichloroethan

4,4-Dichlorodiphenyltrichloroethan (DDT) has a log K_{ow} of 6.91. DDT is almost insoluble at 25 °C. DDT is a chlorinated hydrocarbon and is used as an insecticide. The organochlorine insecticide DDT belongs to CNS (central nervous system) seizure agents interacting with the nervous system causing tremors and convulsions (BARRON *et al.*, 2002). DDT inhibits the Na-channel deactivation (SCHMIDT, 1986) leading to continuous stimulation of nerves of insects, crustaceans and mammals (JCIA, 1997). The usage and production of DDT was prohibited by law in West Germany in 1972. It has been banned because of extreme environmental persistence and extensive biomagnification in the food web. However, DDT is still used against *Anopheles* spp. for malaria control in countries of the Indian subcontinent, Africa, Asia, and South America. The chemical structure is shown in figure 2.10.

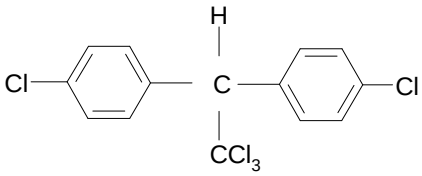


Figure 2.10: Chemical structure of DDT ($C_{14}H_9Cl_5$)

2.2.8 Tributyltinchloride

Tributyltinchloride (TBT-Cl) has a log K_{ow} of 4.7. TBT-Cl has a water solubility of 0.75 $mg\,l^{-1}$ at 25 °C. The chemical structure is shown in figure 2.11. The organotin TBT-Cl

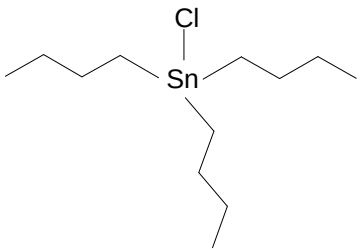


Figure 2.11: Chemical structure of tributyltinchloride ($C_{12}H_{27}ClSn$)

was mainly used as active biocide in antifouling paints for watercraft. The endocrine effects on water organisms are described in literature (STROBEN, 1993; BAUER *et al.*, 1997;

OEHLMANN *et al.*, 1998; BUA, 2003). The organometal compound TBT effects membranes because of its comparatively high hydrophobic, lipid partitioning properties. The toxicity of TBT compounds on various microorganisms is attributed to the inhibition of various enzymes, such as ATPase, NADH oxidase, beta-galactosidase, and alkaline phosphatase (WHITE *et al.*, 1999). The binding of TBT to the androgen receptor is discussed to cause an androgenisation of female prosobranch gastropod (STROBEN, 1993; SANTOS *et al.*, 2005). Further, the inhibition of aromatase by TBT is hypothesized to induce imposex (STROBEN, 1993; BETTIN *et al.*, 1996; SANTOS *et al.*, 2005). Apart from that, OBERDÖRSTER & MCCLELLAN-GREEN (2000) formulate, as a further explanation, the effect of TBT as neurotoxin. BARRON *et al.* (2002) describes the organometal's mode of action as nerve tissues damaging. The usage of organotin antifouling systems has been prohibited worldwide since 2003.

2.3 Acute toxicity tests

Acute toxicity tests with exposition of the selected species via the water phase were conducted. The accomplished data can be used for comparison with acute aquatic toxicity data of other standard test organisms such as algae, daphnia, and fish species. Also, the data can be used as a "range finder" using the equilibrium method for selecting the concentrations within the sediment toxicity tests. Within sediment toxicity tests, the concentration of the substance is measured in the sediment, pore water, and overlying water. The measured concentrations in pore water and overlying water can then be accounted as toxic or nontoxic, referring to the obtained acute toxicity data.

Analytical measurements were done at the Fraunhofer Institute for Molecular Biology and Applied Ecology, Schmallenberg, Germany. For analytical measurements 20 mL were sampled at the beginning and the end of the experiments with the exception of the tests with *C. riparius* where 10 mL were sampled at the end of the test. At the end of the test, samples of the replicates were merged. Detailed description of analytical analysis is summarized in table 2.1.

2.3.1 Acute toxicity test with *L. variegatus* (96 hours)

The acute toxicity test with *L. variegatus* was conducted at 20 °C and a regime of 12 hours of light and 12 hours of dark. Twenty-four well microtiter plates were used as test beakers. Two ml of the test media were placed into each well. Reconstituted water following the ISO-standard 7346-3 was used. The amount of the substances used to prepare the ISO-water was reduced to 1/5 to obtain a hardness of 3 °dH (BACHMANN, 1996; SCHULTE, 1997; OECD, 1998) . Ten replicates were used for each concentration, control, and, if necessary, solvent control. Five concentrations were tested for each test substance. To have organisms of the same developmental and physiological state within the test, adult worms were cut 3 weeks

Table 2.1: Overview of the analytical methods for water samples, if two measurement types or method characteristics are listed, the tests and the analytical procedures were performed in different years using different methods

	TNT	3,4-DCA	2,4-DCP	TBT-Cl	PCP	B(a)P	4,4-DDT	Cd
Method of measurement	GC/MS/MS	GC/MS/MS	GC/MS/MS	GC/AED	GC/MS/MS	IonTrap GC/MS (SIS) or Quadrupole GC/MS (SIM)	GC/MS/MS	graphite furnace-AAS, DIN EN ISO 5961 or ICP-MS following DIN 38406-29
Instrument	Varian Saturn 2000	Varian Saturn 2000	Varian Saturn 2000	Agilent GC 5890 and AED G2350A	Varian Saturn 2000 or FinniganMAT ITS40	Varian Saturn 2000 or Varian 1200L	Varian Saturn 2000 or FinniganMAT ITS40	-
Extraction	toluene, adjustment to pH 7 (phosphate buffer)	toluene, adjustment to pH 9 (phosphate buffer)	toluene, acidification with hydrochloric acid	ethanol / Na-diethyldithiocar-bamate	toluene, acidification with hydrochloric acid	toluene	n-hexane	after acidification
Derivatization agent	-	-	MSTFA	sodium tetraethylborate	MSTFA	-	-	-
Internal Standard	2,6-DNT	2,4-DCA	2,6-DCP	tripropyltin for tributyltin (TBT)*	2,4,6-TBP	B(b)F / B(k)F	2,4-DDT	-
Clean up	-	-	-	adsorption chroma-tography on silica gel	-	-		-
Calibration type	basic and matrix / basic and matrix	basic and matrix / basic	matrix / matrix	matrix	matrix / matrix	matrix / matrix	basic and matrix / matrix	-
Calibration levels (N), curve fit	8, linear / 10, quadratic	10, linear / 10, linear	9, linear / 10, quadratic	9, linear	9, linear / 10, linear	9, linear / 10, quadratic	9, linear / 7, linear	-
Recovery rate [%]	119 / 124	97 / 97	- / -	80 – 110	- / -	97.5 / 100	106 / -	-
Regression coefficient, r	0.9978 / 0.9994	0.9992 / 0.9991	0.9996 / 0.9997	0.9998	0.9989 / 0.9998	0.9975 / 0.9998	0.9951 / 0.9999	-
Relative method standard deviation, V_{x0} [%]	5.55 / 3.02	3.04 / 3.34	1.79 / 1.50	4.1	3.63 / 3.14	4.79 / 1.81	6.69 / 1.16	-
Limit of Quantification, LOQ	0.004 $mg\,l^{-1}$	0.004 $mg\,l^{-1}$	0.004 $mg\,l^{-1}$	0.012 $\mu g\,l^{-1}$ (TBT cation)	5 $\mu g\,l^{-1}$ / 2 $\mu g\,l^{-1}$	4 $\mu g\,l^{-1}$ / 0.02 $\mu g\,l^{-1}$	0.1 $\mu g\,l^{-1}$	approx. 1 $\mu g\,l^{-1}$ referring to Cd

MSTFA = N-Methyl-N-trimethylsilyltrifluoroacetamide, * = tetrapropyltin for tetrabutyltin; diheptyltin for dibutyltin; monoheptyltin for monobutyltin

prior to testing. For posterior fragments, about 7 days are required to regenerate a new head (BALATRE-VELTZ *et al.*, 1999). Effects were documented after 96 hours. Lysis, paralysis, lack of haemolymph circulation, and decoloration of the organism were each defined as lethal effects. Convulsive motion, fragmentation, and deformations were each defined as sublethal effects. For better comparison with *C. riparius*, sublethal effects were not used for the calculation of effect concentrations, because for *C. riparius* lethality was the only observable endpoint by the method used.

2.3.2 Acute toxicity test with *C. riparius* (48 hours)

The acute toxicity test with *C. riparius* was conducted at 20 °C and a regime of 12 hours of light and 12 hours of dark. Twenty-four well microtiter plates were used as test beakers. One ml of the test media was placed into each well. Reconstituted water following ISO-standard 7346-3 was used. The amount of the substances used to prepare the ISO-water was reduced to 1/5 to obtein a hardness of 3 °dH (BACHMANN, 1996; SCHULTE, 1997; OECD, 1998). Ten replicates were used for each concentration, control and solvent control (if necessary). Five concentrations were tested for each test substance. Freshly hatched first instar larvae were used in the test. Effects were documented after 48 hours. Lack of motion after external stimulus was defined as lethal endpoint.

2.3.3 Statistical evaluation of acute toxicity tests

EC_X calculation was done using probit transformation (ToxRat®) or Spearman-Karber method.

2.4 28 d-sediment toxicity tests

The 28 d-sediment toxicity tests are test methods for lipophilic chemicals that adsorb to particulate matter and sediment particles (STRELOKE & KÖPP, 1995; OECD, 2000)). For both sediment toxicity tests, the main objective was to provide a complete food source with the artificial sediment. ÅKERBLOM & GOEDKOOP (2003) showed that C and N from added food, as generally done in standardized test methods (STRELOKE & KÖPP, 1995; RISTOLA, 1995; OECD, 2000), is the major nutrient flux (90 to 94%) for larval *Chironomus*. These results and fatty acid compositions of larvae and food resources imply that, when food is added during the test period, larvae will preferentially feed on the added food. Feeding behavior will thus alter exposure pathways and bioavailability (ÅKERBLOM & GOEDKOOP, 2003).

2.4.1 Sediment toxicity test with *L. variegatus*

First, tests without chemicals were done according to OETKEN *et al.* (2001). Because it was not possible to obtain nearly equal results without mortality, the sediment composition had to be changed. The test was conducted at a constant temperature of 20 $\pm$ 1 °C, and a regime of 16 hours light and 8 hours dark. Quartz sand with a defined grain size ranging from 62 - 2000 μm was used. As for tests with *C. riparius*, the main objective was to use a test system without external feeding during the test and to get high reproduction. The organic carbon within the sediment was made up of α-cellulose and of shredded leaves of nettle (*Urtica dioica*), which were a complete food source for the test organisms. α-cellulose was selected as the source of organic carbon because it is commercially available, consistent from batch to batch, and low in contaminant concentrations (KEMBLE *et al.*, 1999). The amount of α-cellulose and nettle leaves was about 1.5% dry weight (dw), which corresponds to about 1.3% organic matter (OM). Quartz sand and the food source were mixed in dry state. Sediment measuring 40 g was placed into each 250 ml beaker. After application of the test substance (see section 2.4.3, page 15), dechlorinated active carbon-filtered tap water was carefully filled onto the sediment. The ratio of overlying water to sediment was 8:1. To obtain organisms of comparable developmental and physiological state at the beginning of the test, adult worms were cut 3 weeks prior to testing. Posterior fragments require about 7 days to regenerate a new head (BALATRE-VELTZ *et al.*, 1999). Worms were introduced into the test system after a sediment aging period of 2 weeks. Sediment aging was done for two reasons: (1) to get an equilibrium between the freely dissolved substance in overlying and pore water and the particle-bound substance within the sediment, and (2) to condition the food source within the sediment. The overlying water was aerated using a Pasteur pipette (5 to 10 bubbles per second). Dissolved oxygen, pH, and conductivity were measured in all beakers at the start and end of the test. Effects were documented after 28 days. The determined toxicological endpoints were the number of worms and biomass (as dry weight).

A different sediment was used for the test with PCP. This test was performed within the international ring test research and development project of the German Federal Environmental Agency to validate a sediment toxicity test with the endobenthic aquatic oligochaete *L. variegatus*. Results are published by EGELER *et al.* (2005). The resulting OECD draft guideline (OECD, 2006) was published in 2006.

Validity criteria

The following validity criteria had to be fulfilled. Mean reproduction of 100% (meaning a doubling of number of worms after 28 days based on the mean number of all replicates) was required for controls and solvent controls. According to the proposed OECD draft guideline (OECD, 2006), which is currently in the validation process, an increase by a factor 1.8 must be fulfilled. The pH of the overlying water should be between 6 and 9 at the start and the end of the test. The dissolved oxygen concentration in the overlying water should be at least 60% of the air saturation value at the test temperature used at the end of the test.

2.4.2 Sediment toxicity test with *C. riparius*

A slightly changed method than that used by OETKEN *et al.* (2001), which is based on OECD 218 (OECD, 2000), was used to perform the 28 d-sediment toxicity tests with *C. riparius*. The test was conducted at a constant temperature of 20 $\pm$ 1 °C, and a regime of 16 hours light and 8 hours dark. Quartz sand with a defined grain size ranging from 62 - 2000 μm was used. The organic carbon within the sediment was made up of shredded leaves of alder (*A. glutinosa*) and nettle (*U. dioica*), which were a complete food source for the test organisms. The amount of alder and nettle leaves was 2%, which served as a complete food source for the larvae. Quartz sand and food source were mixed in dry state. Sediment measuring 135 g was placed into each 600 ml beaker. After application of the test substance (see section 2.4.3, page 15) 30 ml of dechlorinated active carbon filtered tap water was carefully filled onto the sediment to wet the sediment and its components. After one hour the rest of the overlying water (370 ml) was carefully filled on top of the sediment. The ratio of overlying water to sediment was 4:1. Freshly hatched larvae were exposed after a sediment aging period of 2 weeks. Beakers were checked for emerged midges daily. Sex and dry weight were determined. Development rate, which is the reciprocal of EMT_{50} (time at which 50% of the organisms emerged), and emerging rate were calculated. Dissolved oxygen, pH, and conductivity were measured in all beakers at the start and end of the test.

Validity criteria

The following validity criteria were applied in accordance with the OECD 218 (OECD, 2000). The mortality in the controls must not exceed 30% at the end of the test (see also STRELOKE & KÖPP (1995)). Emergence should occur between 12 and 23 days after their insertion into the test vessels. The mean emergence in controls should be in a range of 50 to 70% (see also STRELOKE & KÖPP (1995)). For validity, focus was placed on emergence the since mortality of the larvae was not examined. At the end of the test, the dissolved oxygen concentration in the overlying water should be at least 60% of the air saturation value at the temperature used. The pH of overlying water must be in the range of 6 to 9 in all test vessels. The water temperature should not differ by more than $\pm$ 1.0 °C among vessels at any time during the test.

For data comparison, it would be beneficial to use the same sediment composition and the same water-to-sediment ratios for the two test organisms. Therefore, the sediment for *L. variegatus* was tested for *C. riparius* on a bigger scale. The sediment contained relatively the same components as for *L. variegatus* (see section 2.4.1). Beakers measuring 2000 ml with sediment to overlying water volume ratio of 1:8 were used. Sediment measuring 270 g that contained the same constituents as for *L. variegatus* was used in each beaker. This test composition was simultaneously compared to the “older” sediment composition, each replicated 5 times.

2.4.3 Application and analysis of the test substances

All organic test substances were dissolved in the solvent acetone to prepare a stock solution, and then were diluted. Deionized water was used as a solvent for cadmiumchloride. Artificial sediments for each beaker were spiked separately with the same volume of solvent containing the appropriate amount of test substance for each concentration level. The volume of solvent used was as much as needed to cover the sediment completely. Beakers of the solvent controls were spiked with the appropriate volume of solvent only. Sediment was mixed thoroughly after application of the solvent. The sediment was completely dried in the air stream of the exhaustion hood. Solvent was totally removed from the sand by this method. Then sediment was mixed again. Overlying water was added very carefully to avoid sediment disturbance. Composition and volumes of sediments and overlying water for each organism were described in detail earlier.

Sediment toxicity tests were replicated six times with the exception of *L. variegatus* sediment toxicity tests with 2,4-DCP, B(a)P, cadmium, TBT and TNT (only one replicate for biological endpoints). Two of these six replicates were analyzed chemically for substances concentration in bulk sediment, overlying water, and pore water. Of these, one beaker was sampled at the start of the exposure when animals were added and one beaker was sampled at test completion after 28 days. The latter beakers contained animals that were not chemically analyzed for body burdens. Analytical measurements for the *L. variegatus* sediment toxicity test with PCP were done at the Institut für Wasserchemie, Technische Universität Dresden, Germany. PCP in water samples was analyzed according to DIN EN 12673 (DIN, 1999) (Method of measurement: GC/ MS, instrument: SHIMADZU (GC-17A, MSD QP5000), extraction solution: n-hexan, derivatization: acetic anhydride, internal standard: 2,4,6-tribromphenol, separate matrix calibrations for 3 concentration ranges (0.5-10, 10-100, 100-1,000 $\mu g\,l^{-1}$, linear regression, $R^2 = 0.9998$, 0.9997, 0.9996, n = 7, n = 6, n = 4, method's coefficient of variation (V_{x0}) = 1.5 %), recovery rate = 105 %, limit of quantification (LOQ, P = 95 %) = 0.52 $\mu g\,l^{-1}$, detection limit (DL, P = 95 %) = 0.14 $\mu g\,l^{-1}$. PCP in sediment samples was analyzed according to a modified method of BAM (2001) (Method of measurement: GC/ MS, instrument: SHIMADZU (GC-17A, MSD QP5000), extraction solution: n-hexan/ acetone, derivatization: acetic anhydride, internal standard: 2,4,6-tribromphenol, separate matrix calibrations for 2 concentration ranges (0.03-1.1, 5-45 $mg\,kg^{-1}$ dw, linear regression, R^2 = 0.9993, 0.9994, n = 11, n = 5, V_{x0} = 1.5 %), recovery rate = 110 %, LOQ (P = 95 %) = 0.03 $mg\,kg^{-1}$, DL (P = 95 %) = 0.001 $mg\,kg^{-1}$. All other analytical measurements were done at the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) in Schmallenberg, Germany. The samples for analytical measurements were sampled at the beginning (start of exposure) and end of the experiment. For analytical measurements of overlaying water 150, 300 mL, for pore water 3, 10 mL, and for bulk sediment 40, 120 g (dw) were sampled in experiments with *L. variegatus*, *C. riparius*, respectively. Detailed description of analytical analysis performed at IME is summarized in table 2.1 on page 11 (for water samples) and table 2.2 (for sediment samples).

Table 2.2: Overview of the analytical methods for bulk sediment samples, if two measurement types or method characteristics are listed, the tests and the analytical procedures were performed in different years using different methods

	TNT	3,4-DCA	2,4-DCP	TBT-Cl	PCP	B[a]P	4,4-DDT	Cd
Method of measurement	HPLC/UV	GC/MS/MS	GC/MS/MS	GC/AED	GC/MS	IonTrap GC/MS (SIS) or Quadrupole GC/MS (SIM)	GC/MS/MS	graphite furnace-AAS, DIN EN ISO 5961 or ICP-MS following DIN 38406-29
Instrument	Gynkotek model 480 / Gynkotek UVD 320	Varian Saturn 2000	Varian Saturn 2000	Agilent GC 5890 and AED G2350A	Varian Saturn 2000 or FinniganMAT ITS40	Varian Saturn 2000 or Varian 1200L	FinniganMAT ITS40	-
Extraction	methanol, ultrasonic extraction	acetone/toluene mixture, adjustment to pH 9, ultrasonic extraction	acetone/n-hexane mixture, acidification with hydrochloric acid	ethanol/Na-diethyldithiocar-bamate	acetone/toluene mixture, acidification with hydrochloric acid	acetone/toluene mixture	acetone/n-hexane mixture	after acidification
Derivatization agent	-	-	MSTFA	sodium tetraethylborate	MSTFA	-	-	-
Internal Standard	none	2,4-DCA	2,6-DCP	tripropyltin for tributyltin (TBT)	2,4,6-TBP	BbF / BkF	2,4-DDT	-
Clean up	-	liquid/liquid extraction, water/toluene	liquid/liquid extraction, water/n-hexane	adsorption chroma-tography on silica gel	liquid/liquid extraction, water/toluene	liquid/liquid extraction, water/toluene	adsorption chroma-tography on silica gel	-
Calibration type	external	matrix	basic / matrix	matrix	matrix / basic	matrix / basic	matrix	-
Calibration levels (N), curve fit	7, linear	8, linear	9, linear / 8, quadratic	9, linear	8, linear / 9, quadratic	9, linear / 10, quadratic	8, linear	-
Recovery rate [%]	> 90	83.6	95.6 / -	80 – 110	99.4 / 103	- / 95.8	-	-
Regression coefficient, r	0.9997 (TNT)	0.9996	0.9996 / 0.9999	0.9999	0.9999 / 1.0000	0.9998 / 0.9998	0.9988	-
Relative method standard deviation, Vx0 [%]	3.59	1.99	1.91 / 0.91	4.1	1.45 / 1.45	1.87 / 1.81	7.16	-
Limit of Quantification LOQ	0.5 $mg\,kg^{-1}$ dw (TNT)	0.1 $mg\,kg^{-1}$ dw	0.25 $mg\,kg^{-1}$ dw	2.4 $\mu g\,kg^{-1}$ dw (TBT cation)	1 $mg\,kg^{-1}$ dw / 0.1 $mg\,kg^{-1}$ dw	0.2 $mg\,kg^{-1}$ dw / 5 $\mu g\,kg^{-1}$ dw	1.5 $\mu g\,kg^{-1}$ dw	approx. 0.03 $mg\,kg^{-1}$ referring to Cd

MSTFA = N-Methyl-N-trimethylsilyltrifluoroacetamide, * = tetrapropyltin for tetrabutyltin; diheptyltin for dibutyltin; monoheptyltin for monobutyltin

2.4.4 Statistical evaluation of sediment toxicity tests

The following procedure was used to set controls, solvent controls, or pooled controls as reference for NOEC testing and computation of EC_X. Data of controls and solvent controls were tested for significant differences by Student-t Test (p = 0.05). Controls and solvent controls were pooled if there was no difference. If there was a difference, computations were done with solvent controls as reference. If not otherwise stated, the EC_X calculation was done using probit transformation. NOEC/LOEC observation was done by using the Williams test ($p<0.05$), if not otherwise stated in the text. The program ToxRat® was used for statistical calculation.

For tests with *L. variegatus* with only one replicate per concentration, a NOEC/LOEC observation was estimated as follows. The LOEC was defined as any difference of the endpoint from the control that is higher than the average coefficient of variance (see equation 2.1) of solvent controls of tests with more than one replicate. The coefficient of variance (*CV*) is described as follows:

$$CV = (s/\bar{X}) \times 100 \tag{2.1}$$

where s equals standard deviation and $\bar{X}$ equals the arithmetic mean.

Within regulatory context, NOEC values are used to derive predictive no effect concentrations (PNECs). The usage of NOEC has been criticized in general (SUTER II, 1996; CHAPMAN *et al.*, 1996; HANSON & SOLOMON, 2002). The use of the EC_{10} as a substitute for the statistical NOEC as a measure of low toxicity has been recommended (CHAPMAN *et al.*, 1996; BAILER & ORIS, 1997). The following procedure is suggested in the technical guidance document on risk assessment (TGD, 2003) if no NOEC is available: "An EC_{10} for a long-term test which is obtained by extrapolation using appropriate statistics (e.g. Probit analysis) can be considered as a NOEC." Differences between NOEC and EC_{10} can have several reasons. NOEC depends on the selected test concentrations and the test systems variability. Test systems (i.e. algae test) with low variability can exhibit significant effects with 5% difference to the control (SHIEH *et al.*, 2001; ISNARD *et al.*, 2001). In test systems with high variability, only effects of 10 to 30% can be attributed as significant (MARCHINI, 2002; MOORE *et al.*, 2004). This is also the case for the sediment toxicity tests with the two invertebrates, for which high mean coefficient of variances were calculated (see details in section 3.9.1 on page 125 and section 3.9.2 on page 129).

3 Results and Discussion

3.1 Acute toxicity tests

This section discusses the results of the analytical measurements performed at the Fraunhofer Institute for Molecular Biology and Applied Ecology in Schmallenberg, Germany. All effect data of acute toxicity tests refer to nominal and effective concentrations. To limit expenses for chemical analysis, only three concentrations of the tested concentrations were analyzed for each test. Effective concentrations for LC_X-values are calculated as follows: If not otherwise stated, LC_X-values were corrected by a relative factor. The arithmetic mean of measured concentrations at the start and end of the test was calculated for each measured concentration. Assuming a first order kinetic degradation/ disappearance process over the test period, a geometric mean was calculated out of these values to get the relative factor by which nominal concentrations were corrected. This procedure was required because not all concentrations were analyzed. It was defined that nominal concentrations were corrected, if analytical findings in test water were out of the range of 80% to 120% of the nominal concentration at the start and end of the exposure period.

3.1.1 Analytical results of acute toxicity tests

No analysis was done for B(a)P since no biological effects were observed in the acute toxicity tests up to nominal concentration of 2 mgl^{-1}.

3.1.1.1 Cadmiumchloride

Results of analytical measurements of cadmium for all acute toxicity tests are shown in table 3.1 on page 20.

3.1.1.1.1 *L. variegatus* acute toxicity test with cadmium The percentage of cadmium of nominal concentrations found in test water at the start of the exposure period ranged from 91% to 99% with a mean of 94% for the three analyzed concentrations, whereas findings decreased to a mean of 45% (minimum of 35% and a maximum of 53%) at the end of the exposure period. The mean analytical findings in test water were higher than 80% of the

nominal concentration at the start of the exposure period. Therefore, the obtained LC_X values based on nominal concentrations were corrected to obtain effective concentrations.

3.1.1.1.2 *C. riparius* acute toxicity test with cadmium

The percentage of cadmium of nominal concentrations found in test water at the start of the exposure period ranged from 97% to 107% with a mean of 101% for the three analyzed concentrations, whereas findings decreased to a mean of 96% (minimum of 89% and a maximum of 104%) at the end of the exposure period. The mean analytical findings in test water were higher than 80% of the nominal concentration at the start of the exposure period. Therefore, the obtained LC_X values based on nominal concentrations were treated as effective concentrations.

Table 3.1: Results of analytical measurements of cadmium of acute toxicity tests, nom. = nominal, conc. = concentration, geo. = geometric

species	nom. conc. $mg\,l^{-1}$	time	analyzed conc. $mg\,l^{-1}$	% of nom.	mean of nom. at start and end of test [%]	geo. mean
C.r.	1.92	t0	1.86	97	101	
		t48h	1.82	95	96	98.7
	3.98	t0	4.00	100		
		t48h	3.56	89		
	7.66	t0	8.17	107		
		t48h	7.96	104		
L.v.	0.18	t0	0.16	91	94	
		t96h	0.06	35	45	65.2
	0.25	t0	0.24	93		
		t96h	0.12	47		
	0.50	t0	0.50	99		
		t96h	0.27	53		

3.1.1.2 Pentachlorophenol

Results of analytical measurements for all acute toxicity tests are shown in table 3.2.

3.1.1.2.1 *L. variegatus* acute toxicity test with PCP

The percentage of PCP of nominal concentrations found in test water of the two analyzed concentrations at the start of the exposure period were 70% and 96% with a mean of 83%. Measured values were 43% and 51% of the nominal concentration at the end of the exposure period. The mean analytical findings in test water were lower than 80% of the nominal concentration at the end of the exposure period. Therefore, the nominal LC_X values were corrected to obtain effective concentrations.

3.1.1.2.2 *C. riparius* acute toxicity test with PCP

The percentage of PCP of nominal concentrations found in test water at the start of the exposure period ranged from 90% to 103% with a mean of 98% for the three analyzed concentrations, whereas findings decreased to a mean of 89% (minimum of 75% and a maximum of 97%) at the end of the exposure period. The mean analytical findings in test water were higher than 80% of the nominal concentration at the start and end of the exposure period. Therefore, the obtained LC_X values based on nominal concentrations are treated as effective concentrations.

Table 3.2: Results of analytical measurements of PCP of acute toxicity tests, nom. = nominal, conc. = concentration, geo. = geometric

species	nom. conc. $mg\,l^{-1}$	time	analyzed conc. $mg\,l^{-1}$	% of nom.	mean of nom. at start and end of test [%]	geo. mean
C.r.	4	t0	4.14	103	98	
		t48h	3.90	97	89	93.5
	1	t0	0.90	90		
		t48h	0.75	75		
	2	t0	2.01	101		
		t48h	1.92	96		
L.v.	0.31	t0	0.22	70	83	
		t96h	0.13	43	47	62.7
	0.42	t0	0.41	96		
		t96h	0.21	51		

3.1.1.3 2,4-Dichlorophenol

Results of analytical measurements for all acute toxicity tests are shown in table 3.3.

3.1.1.3.1 *L. variegatus* acute toxicity test with 2,4-DCP

The percentage of 2,4-DCP of nominal concentrations found in test water of the two analyzed concentrations at the start of the exposure period were 77% and 84% with a mean of 81%. Measured values were 25% and 27% of the nominal concentration at the end of the exposure period. The mean analytical findings in test water were lower than 80% of the nominal concentration at the end of the exposure period. Therefore, the nominal LC_X values were corrected to obtain effective concentrations.

3.1.1.3.2 *C. riparius* acute toxicity test with 2,4-DCP

The percentage of 2,4-DCP of nominal concentrations found in test water at the start of the exposure period ranged from 83% to 90% with a mean of 87% for the three analyzed concentrations, whereas findings decreased to a mean of 21% (minimum of 21% and a maximum of 22%) at the end of the exposure period. The mean analytical findings in test water were lower than 80% of the nominal concentration

at the end of the exposure period. Therefore, the nominal LC_X values were corrected to obtain effective concentrations.

Table 3.3: Results of analytical measurements of 2,4-DCP of acute toxicity tests, nom. = nominal, conc. = concentration, geo. = geometric

species	nom. conc. $mg l^{-1}$	time	analyzed conc. $mg l^{-1}$	% of nom.	mean of nom. at start and end of test [%]	geo. mean
C.r.	4.5	t0	4.0	89	87	
		t48h	1.0	22	21	43.0
	6.75	t0	5.6	83		
		t48h	1.4	21		
	10.125	t0	9.1	90		
		t48h	2.1	21		
L.v.	25	t0	19.3	77	81	
		t96h	6.8	27	26	45.6
	17.7	t0	14.9	84		
		t96h	4.4	25		

3.1.1.4 Tributyltinchloride

Results of analytical measurements of all acute toxicity tests are shown in table 3.4.

3.1.1.4.1 *L. variegatus* acute toxicity test with TBT-Cl The percentage of TBT of nominal concentrations found in test water at the start of the exposure period ranged from 559% to 714% with a mean of 636% for the three analyzed concentrations whereas findings decreased to a mean of 40% (minimum of 29% and a maximum of 49%) at the end of the exposure period. The relatively high concentrations in samples at t0 cannot be explained. Mean analytical findings in test water were higher than 120% of the nominal concentration at the start of the exposure period and below 80% of the nominal concentration at the end of the exposure period. Therefore, the nominal LC_X values were corrected to obtain effective concentrations.

3.1.1.4.2 *C. riparius* acute toxicity test with TBT-Cl The percentage of TBT and its metabolites of nominal concentrations, found in test water at the start of the exposure period ranged from 63% to 80% with a mean of 68% for the three analyzed concentrations, whereas findings decreased to a mean of 39 % (minimum of 31% and a maximum of 43%) at the end of the exposure period. The mean analytical findings in test water were lower than 80% of the nominal concentration at the start and end of the exposure period. Therefore, the nominal LC_X values were corrected to obtain effective concentrations.

Table 3.4: Results of analytical measurements of TBT-Cl of acute toxicity tests, nom. = nominal, conc. = concentration, geo. = geometric

species	nom. conc. $\mu g l^{-1}$TBTCL	nom. conc. $\mu g l^{-1}$TBT	time	measured conc. [$\mu g l^{-1}$] DBT	MBT	TTBT	TBT	TBT in % of nom.	mean of nom. at start and end of test [%]	geo. mean [%]
C.r.	81.3	72.4	t0	1	0.08	0.23	58.02	80.1	68	
			t48h	0.31	0.21	0.1	22.48	31.0	39	51.3
	108.8	97.0	t0	1.43	0.06	0.25	59.54	61.4		
			t48h	0.53	0.32	0.11	41.33	42.6		
	145	129.2	t0	1.99	0.06	0.34	81.33	62.9		
			t48h	0.53	0.35	0.09	54.36	42.1		
L.v.	7.1	6.3	t0	0.82	0.1	0.09	35.36	558.9	636	
			t96h	1.1	1.38	0.05	1.8	28.5	40	159.5
	10	8.9	t0	1.02	0.13	0.15	56.67	636.0		
			t96h	1.84	2.52	0.06	3.78	42.4		
	14.1	12.6	t0	1.24	$<$ lq	0.64	89.72	714.1		
			t96h	3.66	3.03	0.2	6.17	49.1		

lq = limit of quantification, lq = 0.012 $\mu g l^{-1}$

3.1.1.5 2,4,6-trinitrotoluene

Results of analytical measurements for all acute toxicity tests are shown in table 3.5.

3.1.1.5.1 *L. variegatus* acute toxicity test with TNT The percentage of TNT of nominal concentrations found in test water at the start of the exposure period ranged from 109% to 117% with a mean of 114% for the three analyzed concentrations. A mean of 90% (minimum of 75% and a maximum of 102%) was found at the end of the exposure period. The mean analytical findings in test water were within the range of 80% to 120% of the nominal concentration at the start and end of the exposure period. Therefore, the obtained LC_X values based on nominal concentrations are treated as effective concentrations.

3.1.1.5.2 *C. riparius* acute toxicity test with TNT The percentage of TNT of nominal concentrations found in test water at the start of the exposure period ranged from 112% to 116% with a mean of 114% for the three analyzed concentrations. A mean of 114% (minimum of 111% and a maximum of 118%) was found at the end of the exposure period. The mean analytical findings in test water were within the range of 80% to 120% of the nominal concentration at the start and end of the exposure period. Therefore, the obtained LC_X values based on nominal concentrations are treated as effective concentrations.

Table 3.5: Results of analytical measurements of TNT of acute toxicity tests, nom. = nominal, conc. = concentration, geo. = geometric

species	nom. conc. $mg\,l^{-1}$	time	analyzed conc. $mg\,l^{-1}$	% of nom.	mean of nom. at start and end of test [%]	geo. mean
C.r.	10	t0	11.53	115	114	
		t48h	11.11	111	114	114.1
	14.14	t0	15.82	112		
		t48h	15.79	112		
	20	t0	23.23	116		
		t48h	23.69	118		
L.v.	7.07	t0	8.11	115	114	
		t96h	5.29	75	90	101.0
	10	t0	11.71	117		
		t96h	9.21	92		
	14.14	t0	15.40	109		
		t96h	14.49	102		

lq = limit of quantification, lq = 0.004 $mg\,l^{-1}$

3.1.1.6 4,4-Dichlorodiphenyltrichloroethan

Results of analytical measurements for all acute toxicity tests are shown in table 3.6.

3.1.1.6.1 *L. variegatus* acute toxicity test with DDT The percentage of DDT of nominal concentrations found in test water at the start of the exposure period ranged from 0.5% to 1.1% with a mean of 0.7% for the three analyzed concentrations. A mean of 0.2% (minimum of 0.1% and a maximum of 0.3%) was found at the end of the exposure period. The mean analytical findings in test water were out of the range of 80% to 120% of the nominal concentration at the start and end of the exposure period. Therefore, the nominal LC_X values were corrected to obtain effective concentrations.

3.1.1.6.2 *C. riparius* acute toxicity test with DDT The percentage of DDT of nominal concentrations found in test water at the start of the exposure period were 0.4% and 1.4% of nominal concentrations. The third analyzed concentration was below the limit of quantification. Of the nominal concentration, 36% were analyzed in the 0.03 $mg l^{-1}$ treatment at the end of the exposure period. Values were below the limit of quantification in the lower analyzed concentrations. The mean analytical findings in test water were lower than 80% of the nominal concentration at the start and end of the exposure period. Therefore, the nominal LC_X values were corrected to obtain effective concentrations.

Table 3.6: Results of analytical measurements of DDT of acute toxicity tests, nom. = nominal, conc. = concentration, geo. = geometric

species	nom. conc. $mg l^{-1}$	time	analyzed conc. $\mu g l^{-1}$	% of nom.	mean of nom. at start and end of test [%]	geo. mean
C.r.	0.009	t0	0.12	1.4	0.9	
		t48h	< lq	-	36.0	5.6
	0.0165	t0	< lq	-		
		t48h	< lq	-		
	0.03	t0	0.11	0.37		
		t48h	10.81	36		
L.v.	0.56	t0	5.90	1.05	0.7	
		t96h	0.54	0.10	0.2	0.4
	1.125	t0	5.81	0.52		
		t96h	2.97	0.26		
	2.25	t0	14.97	0.67		
		t96h	4.54	0.20		

lq = limit of quantification, lq = 0.1 $\mu g l^{-1}$

3.1.1.7 3,4-Dichloroaniline

Results of analytical measurements of all acute toxicity test are shown in table 3.7.

3.1.1.7.1 *L. variegatus* acute toxicity test with 3,4-DCA The percentage of 3,4-DCA of nominal concentrations found in test water at the start of the exposure ranged from 90% to 100% with a mean of 94% for the three analyzed concentrations. A mean of 36% (minimum of 32% and a maximum of 40%) was found at the end of the exposure period. The mean analytical findings in test water were higher than 80% of the nominal concentrations at the start of the exposure period, but lower than 80% at the end of the exposure period. Therefore, the nominal LC_X values were corrected to obtain effective concentrations.

3.1.1.7.2 *C. riparius* acute toxicity test with 3,4-DCA The percentage of 3,4-DCA of nominal concentrations found in test water at the start of the exposure was 91% in the analyzed concentration of 2.5 mgl^{-1}. A mean of 43% (minimum of 38% and a maximum of 53 %) was found at the end of the exposure period. The mean analytical findings in test water were higher than 80% of the nominal concentration at the start of the exposure period, but lower than 80% at the end of the exposure period. Therefore, the nominal LC_X values were corrected to obtain effective concentrations.

Table 3.7: Results of analytical measurements of 3,4-DCA of acute toxicity tests, nom. = nominal, conc. = concentration, geo. = geometric

species	nom. conc. mgl^{-1}	time	analyzed conc. mgl^{-1}	% of nom.	mean of nom. at start and end of test [%]	geo. mean
C.r.	10	t0	n.a.	-	91	
		t48h	3.80	38	43	62.6
	20	t0	n.a.	-		
		t48h	10.63	53		
	2.5	t0	2.27	91		
		t48h	0.96	38		
L.v.	10	t0	10.03	100	94	
		t96h	3.51	35	36	58.0
	20	t0	18.61	93		
		t96h	8.00	40		
	5	t0	4.51	90		
		t96h	1.58	32		

n.a. = not analyzed

3.1.2 Overview of acute toxicity data

To limit expenses for chemical analysis, only three concentrations of the tested concentrations were analyzed for each test. The method for calculation of effective concentrations for LC_X-values is described in the beginning of section 3.1 on page 19.

Nominal and effective data for acute toxicity tests with the tested invertebrates are shown in table 3.8. Additionally, table 3.8 shows mean LC_{50} literature data of *D. magna* (juvenile, 48-hour, without feeding). Data of juvenile *D. magna* of studies without feeding were used to have comparable data of similar test conditions with test organisms of the same species life stage. Most data were available for the juvenile life stage.

3.1.2.1 Acute toxicity data of *L. variegatus*

Calculations of effective LC_X values were done for all tests except for the test with TNT. B(a)p was tested up to 2 $mg l^{-1}$, which is far above the solubility level. No effects were observed up to this concentration. The following comparisons are based on effective concentrations. The most toxic substance is DDT followed by TBT, PCP, and cadmium. PCP and cadmium are of equal toxicity. Then follows TNT and 3,4-DCA. The least toxic of the tested substances is 2,4-DCP. The selected substances cover a wide range of toxicity. LC_{50} values of the most and the least toxic substance are by a factor of 7600 different (based on $\mu mol l^{-1}$).

3.1.2.2 Acute toxicity data of *C. riparius*

Calculations of effective LC_X values were done for tests with 3,4-DCA, DDT, 2,4-DCP and TBT. B(a)p was tested up to 2 $mg l^{-1}$, which is far above the solubility level. No effects were observed up to this concentration. The following comparisons are based on effective concentrations. The most toxic substance is DDT followed by TBT, PCP, 2,4-DCP, 3,4-DCA, cadmium, and TNT. The selected substances cover a wide range of toxicity. LC_{50} values of the most and the least toxic substance differ by a factor of 24,000 (based on $\mu mol l^{-1}$).

Sensitivity of different larval stages of *C. riparius*

Evidence from acute tests suggests that the first instar larvae are more sensitive than older larval stages. As outlined by NAYLOR & HOWCROFT (1997), differences in sensitivity seem to be highly chemical-specific. For example, when tested with cadmium, the 24-hour LC_{50} for the first instar was 952 less than that for the fourth instar (WILLIAMS *et al.*, 1986), but for TBT the 48-hour LC_{50} for first instar of this study was the same as that for fourth instar (HAHN & SCHULZ, 2002). For nickel (48-hour EC_{50}) the ratio of second to first instar was only 2.1 (POWLESLAND & GEORGE, 1986) and for copper (96-hour EC_{50}), the ratio was 2.6 (NEBEKER *et al.*, 1984).

3.1.3 Comparative discussion of acute toxicity data

In the first part of the discussion of acute toxicity data, the reported values of this study are compared and discussed with literature data. *C. riparius* was less sensitive to TBT than *L.*

Table 3.8: Overview of acute toxicity data of tested invertebrates, chemicals are ranked according to sensitivity of *L. variegatus* (based on effective concentrations, nominal equals effective concentration if nominal concentration is given only, lower and upper confidence limits are given in parenthesis)

	L. variegatus LC_{50} (96 h)		*C. riparius* (1^{st} instar) LC_{50} (48 h)		*D. magna* * LC_{50} ($\bar{x}$)	
Chemical	(mgl^{-1})	($\mu moll^{-1}$)	(mgl^{-1})	($\mu moll^{-1}$)	(mgl^{-1})	($\mu moll^{-1}$)
DDT	0.70 (0.41-1.06)	2.0 (1.2-3.0)	0.016 (0.012-0.024)	0.045 (0.34-0.068)	0.003 a	0.008 a
DDT eff.	0.003 (0.002-0.004)	0.008 (0.005-0.012)	0.0009 (0.0007-0.0013)	0.0025 (0.0019-0.0038)		
TBT-Sn	0.0040 (0.0037-0.0047)	0.034 (0.031-0.040)	0.048 (0.041-0.07)	0.40 (0.34-0.59)	0.0022 b	0.019 b
TBT-Sn eff.	0.0064 (0.0059-0.0075)	0.054 (0.050-0.063)	0.025 (0.021-0.036)	0.21 (0.18-0.30)		
Cd	0.31 (0.27-0.36)	2.8 (2.4-3.2)	4.8 (3.5-6.4)	42 (31-57)	0.07 c	0.61 c
Cd eff.	0.2 (0.18-0.23)	1.8 (1.6-2.0)				
PCP	0.34 (0.31-0.37)	1.28 (1.16-1.39)	1.51 (1.18-1.92)	5.6 (4.4-7.2)	0.49 d	1.86 d
PCP eff.	0.21 (0.19-0.23)	0.8 (0.73-0.87)				
B(a)P					0.04 e	0.16 e
B(a)P eff.						
TNT	9.0 (7.0-10.9)	39.8 (30.7-47.8)	13.7 (12.5-15.2)	60.5 (54.8-66.8)	6.6 f	29.1 f
TNT eff.						
3,4-DCA	12.3 (10.3-14.7)	76.0 (63.8-90.5)	9.8 (6.9-13.8)	60 (43-85)	0.19 g	1.1 g
3,4-DCA eff.	7.1 (6.0-8.5)	44 (37-53)	6.1 (4.3-8.6)	38 (27-53)		
2,4-DCP	21.8 (19.0-24.9)	134 (117-153)	7.2 (6.4-8.1)	44.3 (39.7-49.4)	2.6 h	16.0 h
2,4-DCP eff.	9.9 (8.7-11.4)	61 (53-70)	3.1 (2.8-3.5)	19 (17-21)		

* = from literature, juvenile, 48 h, without feeding, eff. = effective concentration,

a = ZIEGENFUSS *et al.* (1986), b = MEADOR (1986), 96-hour exposure with adult daphnids,

c = ATTAR & MALY (1982); BODAR *et al.* (1990); CANTON & SLOOFF (1982); ENSERINK *et al.* (1990); FARGASOVA (1994); GALE *et al.* (1992); HATAKEYAMA & SUGAYA (1989), LEWIS & WEBER (1985); LEWIS & HORNING II (1991); MOUNT & NORBERG (1984); PENTTINEN *et al.* (1998); SCHUYTEMA *et al.* (1984); SPEHAR & CARLSON (1984); TAYLOR *et al.* (1998),

d = ADEMA (1978); ADEMA & VINK (1981); CANTON & ADEMA (1978); FERRANDO *et al.* (1992); HERMENS *et al.* (1984); LEBLANC (1980); LIBER & SOLOMON (1994), MOUNT & NORBERG (1984); OIKARI *et al.* (1992); THURSTON *et al.* (1985)

e = LC_{50} not available instead an EC_{50} (24 h, can be counted lethal) was used (WERNERSSON & DAVE, 1997), f = LIU *et al.* (1976),

g = ADEMA (1978); ADEMA & VINK (1981); FERRANDO *et al.* (1992); MAAS-DIEPEVEEN & LEEUWEN (1986), h = LEBLANC (1980)

variegatus and *H. azteca* (LC_{50} = 2.6 $\mu g l^{-1}$ TBT-Sn (FIEDLER, personal communication)). For *C. riparius*, the value for TBT-Sn coincides with the findings of HAHN & SCHULZ (2002), who found a 48-hour LC_{50} of 25 $\mu g l^{-1}$ (20-30 $\mu g l^{-1}$ 95% confidence intervals) for mid-fourth–stage chironomid larvae. This suggests no difference in sensitivity between the first (in this study) and fourth instar of this species when exposed to TBT.

Whereas WILLIAMS *et al.* (1986) found major differences in sensitivity of larval stages of *C. riparius* when exposed to cadmium. LC_{50} for the first instar was lower by factor 952 than LC_{50} for the fourth instar larvae after a 24-hour exposure. The LC_{50} value for cadmium (4.8 $mg l^{-1}$) of this study coincides with the 24-hour LC_{50} of 2.1 $mg l^{-1}$ for first instar larvae found by WILLIAMS *et al.* (1986). MILANI *et al.* (2003) reported a 96-hour LC_{50} of 2.1 $\mu g l^{-1}$ for the first instar larvae in a test with a silica sand layer plus feeding during exposure. It was stated that problems associated with using first instar chironomids (i.e., abrasion by silica sand on larvae) could have resulted in an overestimation of toxicity. In a 10-day toxicity test with *L. variegatus* (with water exposure and without feeding), an LC_{50} of 158 $\mu g l^{-1}$ were found respectively (PHIPPS *et al.*, 1995) and were in good agreement with the LC_{50} (96 h) of this study. *L. variegatus* of this study was 3 times more sensitive than *Tubifex tubifex* (LC_{50} = 0.87 $mg l^{-1}$ (MILANI *et al.*, 2003)). For *H. azteca*, 72- to 96-hour LC_{50} values in the range from 1.9 to 13 $\mu g l^{-1}$ were reported in literature (NEBEKER *et al.*, 1986; COLLYARD *et al.*, 1994; MILANI *et al.*, 2003; GUST, 2006; FIEDLER, personal communication). Thus, *H. azteca* was up to 2,500 and up to 105 times more sensitive than *C. riparius* and *L. variegatus*, respectively.

96-hour LC_{50} of PCP for *L. variegatus* found by EWELL *et al.* (1986) is 16 times higher (3.2 $mg l^{-1}$ at pH = 7.4 with juvenile worms) than in this study, Whereas HICKEY & MARTIN (1995) calculated a 96-hour LC_{50} of 0.69 $mg l^{-1}$ PCP, which is higher by a factor of 3 than in this study. LC_{50} of PCP for *C. riparius* in this study coincided with the values for fourth instar larvae of 1.948 $mg l^{-1}$ at pH 9 and 0.465 $mg l^{-1}$ at pH 6 (FISHER & WADLEIGH, 1986). In our experiment, mean pH was 7.2. At higher pH, PCP is completely ionized and bears a negative charge. Thus, penetration through membranes is reduced leading to lower toxicity. The 48-hour LC_{50} for *L. variegatus* and fourth instar *C. riparius* of 0.143 and 0.898 $mg l^{-1}$ PCP (at pH 6.5) respectively (KUKKONEN, 2002) are in good agreement with the values of this study. FIEDLER (personal communication) reported a LC_{50} (96 h) of 0.087 $mg l^{-1}$ for *H. azteca* indicating higher sensitivity of *H. azteca* to PCP than *L. variegatus* and *C. riparius*.

C. riparius (second instar) was found to have a 48-hour LC_{50} of 14.8 $mg l^{-1}$ 3,4-DCA (TAYLOR *et al.*, 1991), which is higher by a factor of 2 than the LC_{50} for first instar of this study, suggesting an increasing sensitivity of younger larvae. 48-hour LC_{50} values of 9.2 $mg l^{-1}$ for first instar larvae of *C. riparius* and 11.7 $mg l^{-1}$ reported by OETKEN *et al.* (2001) are in good agreement with the values of this study. When compared to *Gammarus pulex* (48-hour LC_{50} of 17.4 $mg l^{-1}$ 3,4-DCA (TAYLOR *et al.*, 1991)), *C. riparius* and *L. variegatus* are more sensitive. The LC_{50} (96 h) of 11.6 $mg l^{-1}$ for *H. azteca* (FIEDLER, personal communication) is in the same range.

For DDT, 48-hour LC_{50} for *C. riparius* are similar to 10-day LC_{50} of 1.23 $\mu g l^{-1}$ observed

for *Chironomus tentans* (larvae were fed during the test, monolayer of sand was used as substrate) (PHIPPS *et al.*, 1995). LC_{50} (48 hour) of 1 $\mu g l^{-1}$ for second instar *C. tentans* (10-14d) (ZIEGENFUSS *et al.*, 1986) was in good agreement with the value for the first instar *C. riparius* of this study. For DDT, acute toxicity 96-hour LC_{50} values for the amphipods *Gammarus fasciatus* and *Gammarus lacustris* in static tests ranged from 1 to 9 $\mu g l^{-1}$ DDT (SANDERS, 1969, 1972; NEBEKER & GAUFIN, 1964), which is in the range of LC_{50} values for *C. riparius* and *L. variegatus* of this study (0.9 and 3 $\mu g l^{-1}$). 96-hour LC_{50} calculations for *H. azteca* ranged from 0.07 to 0.5 $\mu g l^{-1}$ (HOKE *et al.*, 1994; PHIPPS *et al.*, 1995; LOTUFO *et al.*, 2000; FIEDLER, personal communication). These findings indicate that *H. azteca* is more sensitive to DDT than *C. riparius* and *L. variegatus*.

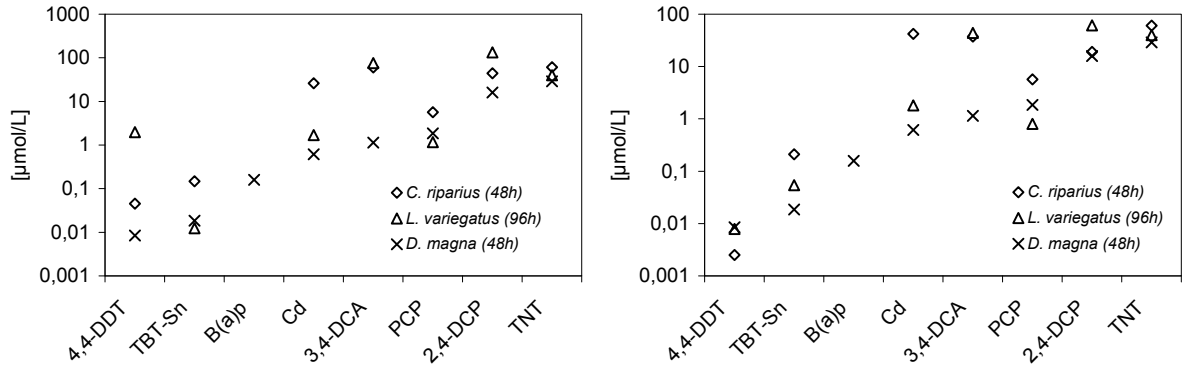
For TNT, 48-hour LC_{50} values ranged from 4.9 to 6.5 $mg l^{-1}$ TNT for *L. variegatus* and *H. azteca* (BAILEY & LIU (1980) in U.S. EPA's AQUIRE/ECETOX database). Further, a 48-hour LC_{50} for *L. variegatus* of 5.2 $mg l^{-1}$ TNT was calculated by LIU *et al.* (1983). These values are only 2 times lower than for *C. riparius* and *L. variegatus* and are expected to lie within interlaboratory variability. FIEDLER (personal communication) calculated a LC_{50} (96 h) of 2.9 $mg l^{-1}$, which is 3 to 4 times lower than values for *L. variegatus* and *C. riparius*.

For B(a)P and *C. riparius* an EC_{50} (24 h) of higher than 5 $\mu g l^{-1}$ B(a)P was found (LYDY *et al.* (1990) in the U.S. Environmental Protection Agency AQUIRE/ECETOX database). Further, a LC_{50} (96 h) of 0.58 $mg l^{-1}$ was reported for *H. azteca* (FIEDLER, personal communication). This was the only information achievable on B(a)P.

Nevertheless, data set for the selected substances and organisms is rare; therefore, acute toxicity tests had to be performed. No data was available for 2,4-DCP and the selected invertebrates. The LC_{50} (96 h) of 2.7 $mg l^{-1}$ (FIEDLER, personal communication) is in good agreement with the value for *C. riparius* indicating equal sensitivity to 2,4-DPC of the two organisms.

LC_{50} values of the two invertebrates based on nominal concentrations plus mean LC_{50} data of *D. magna* (from literature, juvenile, 48-hour, without feeding) are shown in figure 3.1(a). Data of juvenile *D. magna* of studies without feeding were used to have comparable data of similar test conditions with test organisms of the same species life stage. Most data were available for juvenile life stage. The tabulated data were not intended to be a comprehensive compilation of all available data, but were provided as a summary of reviewed literature data. In figure 3.1(b) the LC_{50} values based on effective concentrations are shown.

Even though the loss in swimming activity of *D. magna* is defined as a lethal endpoint, it is sometimes counted as sublethal, resulting in reported EC_X values. For B(a)P and *D. magna* an LC_{50} (48 h) is not available; therefore, an EC_{50}(24 h) that can be counted as LC_{50} was used. Substances were ranked by sensitivity of *D. magna*. The following similarities were observed for the three invertebrates. TBT and DDT were the most toxic, whereas TNT and 2,4-DCP were the least toxic substances for all three organisms. For all organisms, PCP showed intermediate toxicity. No general statement can be made for the other substances.



(a) based on nominal concentrations (b) based on effective concentrations

Figure 3.1: LC_{50} of *D. magna* (48 h), *C. riparius* (48 h) and *L. variegatus* (96 h), substances are ranked by sensitivity of *D. magna*

As described above, there are large differences in the effect concentrations of the selected substances. The ranking for the other organisms are different. Following, substances are ranked by difference in species sensitivity. It was defined that, no or very small difference is observed when the factor of difference in species sensitivity (f_{dss}) is lower or equal to 5. Factors higher than 5 but smaller than 10 are considered a small difference. Values above 10 are considered a large difference. There are similarities in species sensitivity for TNT with f_{dss} = 2.1 based on both nominal and effective concentrations. A difference by a factor higher than 5 but lower than 10 is seen for 2,4-DCP (f_{dss} = 8.4) based on nominal concentrations. A factor lower than 5 (f_{dss} = 3.8) was observed when comparing effective concentrations. Based on effective concentrations, f_{dss} for PCP and TBT were lower than 10 but higher than 5. For DDT, similarities in species sensitivity (f_{dss} = 3.4) were observed when comparing effective concentrations. Differences were the largest for DDT when comparing nominal concentrations (f_{dss} = 232). This is due to testing above water solubility level in the test with *L. variegatus*. For 3,4-DCA and Cd, differences between the lowest and highest value are higher than factor 10, which are considered as large differences according to the definition given above. The largest differences in species sensitivity for both nominal and effective concentrations were observed for cadmium with an f_{dss} of 42. Only, for the organic chemicals with log K_{ow} smaller or equal 3.0 (TNT and 2,4 DCP), differences in sensitivity are smaller than factor 5. This observation does not apply to 3,4-DCA (f_{dss} = 67 for nominal and f_{dss} = 39 for effective concentrations) and DDT (f_{dss} = 232 for nominal and f_{dss} = 3.4 for effective concentrations).

LC_{50} values were lower for chemicals with higher log K_{ow} values indicating that toxicity is dependent on log K_{ow} (see figure 3.2). But toxicity is dependent on the substance dose. Higher lipophilicity results in higher bioconcentration factors, thus resulting in higher inner concentrations in the organisms at relatively lower concentrations in the surrounding water. The lethal body residues at which death is observed are dependent upon chemicals class, mode of action, environmental factors and biological factors such as biotransformation and lipid content (BARRON *et al.*, 2002, and references therein).

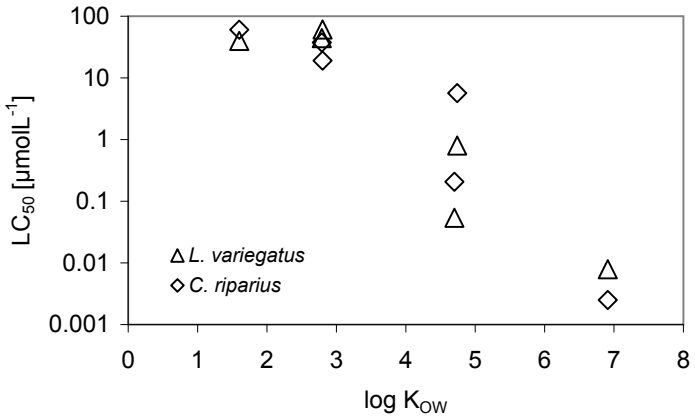


Figure 3.2: LC_{50} of *C. riparius* (48 h) and *L. variegatus* (96 h) in comparison to log K_{ow}

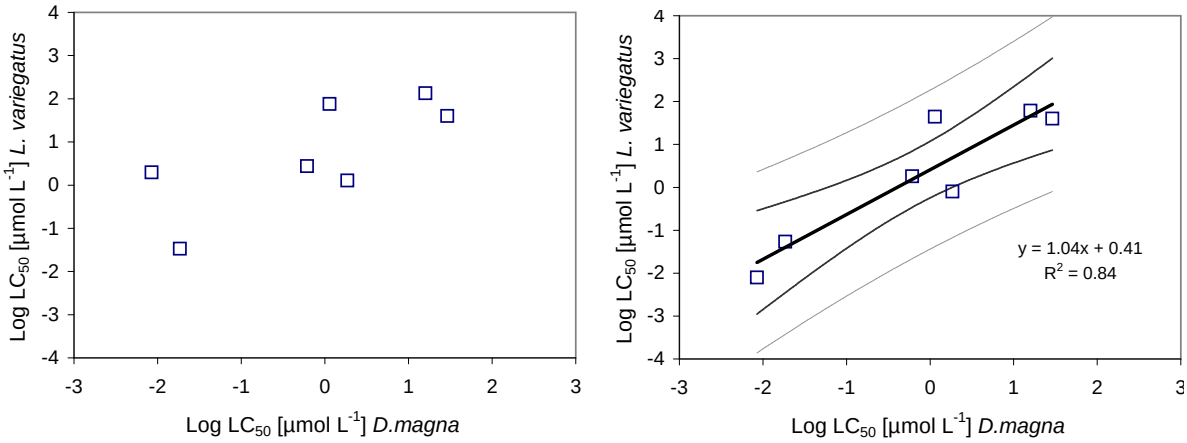
Comparison of LC_{50} values for *C. riparius*, *L. variegatus*, and *D. magna* for the tested chemicals indicates there was no species consistently most sensitive to the eight chemicals. Based on effective concentrations, *D. magna* was the most sensitive species for TBT-Sn, cadmium, and 3,4-DCA. *C. riparius* was the most sensitive species for DDT. *L. variegatus* was the most sensitive species for PCP. 2,4-DCP was equally toxic to *C. riparius* and *D. magna*. 3,4-DCA was equally toxic to *C. riparius* and *L. variegatus*. Values ranged from 60 to 76 $\mu mol l^{-1}$ (38 to 44 $\mu mol l^{-1}$ based on effective concentrations). Whereas *D. magna* (mean LC_{50} = 1.1 $\mu mol l^{-1}$) was more sensitive by a factor of 55 to 66 (33 to 40 based on effective concentrations) than *C. riparius* and *L. variegatus*.

Interspecies correlation of acute toxicity data

WEYERS *et al.* (2000) and LICHT *et al.* (2004) have correlated EC_{50}/ LC_{50} of algae, daphnids, and fish with each other, finding the best correlation parameters between EC_{50} data for *Daphnia* and LC_{50} data for fish (R^2 = 0.6 and 0.79 from linear regression analysis with log transformed data). Due to physiological similarities of the invertebrates, correlation between each other is expected to be significant. The responses of the tests with *C. riparius* and *L. variegatus* were compared with *D. magna* in order to evaluate the ability of the *D. magna* acute toxicity test to predict the response of the others. Therefore, literature data of *D. magna* and the experimental data of the two invertebrates were analyzed for significant correlation. First, the data were tested for normal distribution using the Shapiro-Wilk test. Because the data were not bivariate normally distributed, the Spearman's rank correlation test was used to assess significant correlation between the variables. Data were not transformed before the correlation analysis. It needs to be pointed out that the data set of only eight substances is very small.

Mean 48-hour LC_{50} literature data of *D. magna* are correlated with 96-hour LC_{50} data of *L. variegatus* (see figure 3.3). There is no significant correlation between the *D. magna* and *L. variegatus* data based on nominal concentrations ($p > 0.05$). Significant correlation was

observed when comparing effective concentrations (ρ (Spearman rho) = 0.75, p = 0.033), thus substances, which are toxic for *D. magna*, are also toxic for *L. variegatus*. For data based on effective concentrations, linear regression analysis was performed for log transformed data of *D. magna* and *L. variegatus* because of significant correlation between the data and the fact that conditions were fulfilled for logarithmized data to perform a linear regression analysis (see figure 3.3(b)). The logarithmized data based on nominal concentrations were not bivariate normally distributed. Therefor, a linear regression analysis was not performed. In conclusion,



(a) based on nominal concentration

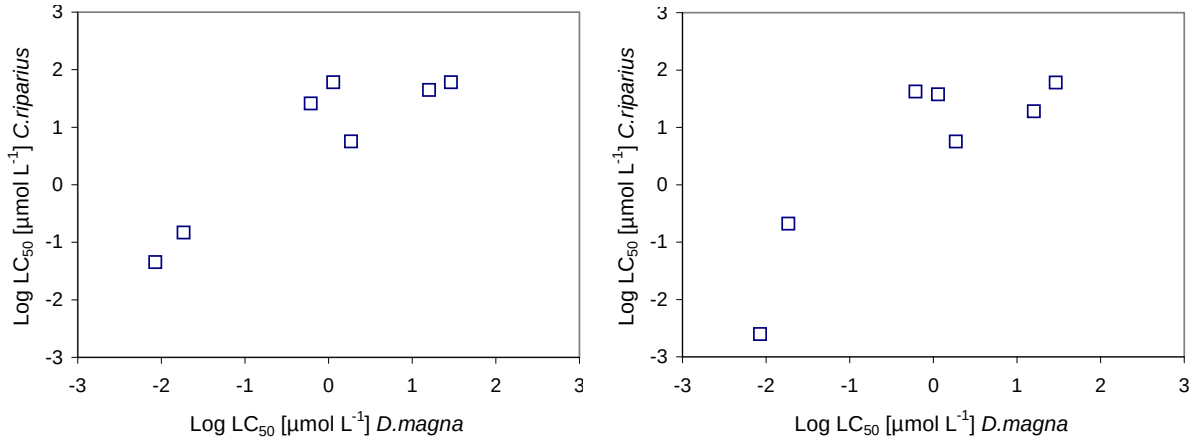
(b) based on effective concentration

Figure 3.3: Correlation of mean 48-hour LC_{50} of *D. magna* (juvenile) with 96-hour LC_{50} of *L. variegatus*, grey line = 95% confidence (line), light grey line = 95% confidence (data)

it can be said that a prediction of toxicity for *L. variegatus* may be possible with *D. magna* data based on effective data only.

Mean 48-hour LC_{50} literature data of *D. magna* are correlated with 48-hour LC_{50} of *C. riparius* (figure 3.4). The LC_{50} data of *D. magna* significantly correlate with LC_{50} data of *C. riparius* (ρ = 0.82/ 0.68 for nominal/ effective concentrations, $p \leq 0.05$). The logarithmized data based on nominal and effective concentrations were not bivariate normally distributed. Therefor, a linear regression analysis was not performed. However, a prediction of toxicity data for *C. riparius* from *D. magna* data is not meaningful due to the small data set of only eight substances and the scattering of the data. The lack of data in the range from 0.02 to 0.6 μmoll^{-1} for *D. magna* data (effective) may also lead to the significant correlations.

In general, the data of *D. magna* significantly correlate with effective data of the two tested invertebrates. Further, FIEDLER (personal communication) showed, that data of *D. magna* significantly ($p \leq 0.05$) correlate with data of *H. azteca* for the same test substances. But extrapolation to other chemicals and the prediction of toxicity for the two invertebrates from *D. magna* data is questionable due to the small data set and the high variation in sensitivity of the test organisms. The substances used in this study not only covered a wide range of lipophilicity but belonged to different mode of action and chemical classes. Substance specific



(a) based on nominal concentrations (b) based on effective concentrations

Figure 3.4: Correlation of 48-hour LC_{50} of *D. magna* with 48-hour LC_{50} of *C. riparius* (1^{st} instar)

mode of action must be taken into account for further data comparison. Data of only one chemical class and specific mode of action may lead to better correlation parameters.

3.2 28-d Sediment toxicity tests - Analytical results

To limit expenses for chemical analysis, only 3 concentrations of the tested concentrations were analyzed for each test. Criteria for handling analytical measurements and its influences on effective concentrations are described as follows. If analytical measurements of bulk at start and end of the exposure period were within ± 20% of nominal concentrations, effective concentrations were accounted as effective concentrations. Otherwise, EC_X and NOEC/LOEC values were corrected by recalculation. For all tests with measured concentrations that differed ± 20% from nominal concentrations, EC_X and NOEC/LOEC were corrected and recalculated as described in detail in section 3.3.9 on page 82.

3.2.1 Pentachlorophenol

3.2.1.1 *C. riparius* sediment toxicity test with PCP

Results of analytical measurements are shown in table 3.9 on page 36. The percentage of nominal concentrations found in bulk at the start of the exposure period ranged from 76% to 85% with a mean of 82% for the three analyzed concentrations whereas findings decreased to a mean of 69% (minimum of 58% and a maximum of 77%) at the end of the exposure period. The mean analytical findings in bulk sediment were higher than 80% of the nominal spiked concentrations at the start of the exposure period but lower than 80% at the end of the exposure

period. Therefore, nominal EC_X and NOEC/LOEC values were corrected and recalculated to obtain effective concentrations.

3.2.1.2 *L. variegatus* sediment toxicity test with PCP

Results of analytical measurements are summarized in section 3.7.1.1.2 on page 112.

3.2.2 2,4-Dichlorophenol

3.2.2.1 *C. riparius* sediment toxicity test with 2,4-DCP

Results of analytical measurements are shown in table 3.10 on page 36. The percentage of 2,4-DCP of nominal concentrations found in bulk at the start of the exposure period ranged from 36% to 52% with a mean of 45% for the three analyzed concentrations. A mean analytical value of 21% (minimum of 14% and a maximum of 24%) of nominal concentrations was found at the end of the exposure period. The mean analytical findings in bulk sediment were lower than 80% of the nominal spiked concentrations at the start of the exposure period. Therefore, calculated nominal EC_X and NOEC/LOEC values were corrected and recalculated to obtain effective concentrations.

3.2.2.2 *L. variegatus* sediment toxicity test with 2,4-DCP

Results of analytical measurements are shown in table 3.10 on page 36. A mean value of 5% (minimum of 4% and a maximum of 6%) of nominal concentrations was found in bulk at the start of the exposure period. The analytical measurements of the lowest concentration tested was below the limit of quantification of 0.25 $mg\,kg^{-1}$ at the end of the exposure period. A mean analytical value of 2% of nominal concentrations was found at the end of the exposure period. The mean analytical findings in bulk sediment were lower than 80% of the nominal spiked concentrations at the start of the exposure period. Therefore, calculated nominal EC_X and NOEC/LOEC values were corrected and recalculated to obtain effective concentrations.

3.2.3 Tributyltinchloride

3.2.3.1 *C. riparius* sediment toxicity test with TBT-Cl

Results of analytical measurements are shown in table 3.11 on page 38. The percentage as a sum of TBT and its metabolites of nominal concentrations found in bulk at the start of the exposure period ranged from 81% to 108% with a mean of 91% for the three analyzed concentrations, whereas findings decreased to a mean of 79% (minimum of 66% and a maximum

Table 3.9: Results of analytical measurements of PCP of the different compartments of sediment toxicity tests

species	nominal $mg\,kg^{-1}$	time	BU $mg\,kg^{-1}$	BU in % of nominal	OW $mg\,l^{-1}$	PW $mg\,l^{-1}$	amount in water mg	% in water of total	amount in sed mg	% in sediment of total	balance $mg\,kg^{-1}$	% of nominal
C.r.	0.5	t0	0.417	83	0.015	0.021	0.01	9	0.06	91	0.46	92
		t28	0.365	73	0.019	0.010	0.01	13	0.05	87	0.42	84
	5	t0	3.806	76	0.202	0.069	0.08	13	0.51	87	4.37	87
		t28	3.839	77	0.136	0.247	0.05	9	0.52	91	4.23	85
	500	t0	426.314	85	32.975	54.067	12.74	18	57.6	82	520.69	104
		t28	288.290	58	50.989	78.461	19.65	34	38.9	66	433.85	87

lq = limit of quantification, lq for bulk = 0.1 $mg\,kg^{-1}$, lq for water = 0.002 $mg\,l^{-1}$

Table 3.10: Results of analytical measurements of 2,4-DCP of the different compartments of sediment toxicity tests

species	nominal $mg\,kg^{-1}$	time	BU $mg\,kg^{-1}$	Bulk in % of nominal	OW $mg\,l^{-1}$	PW $mg\,l^{-1}$	amount in water mg	% in water of total	amount in sed mg	% in sediment of total	balance $mg\,kg^{-1}$	% of nominal
C.r.	17.89	t0	6.45	36	0.61	3.34	0.26	23	0.87	77	8.4	47
		t28	4.15	23	n.a.	0.87	*0.01*	*2*	*0.56*	*98*	*4.2*	*24*
	40	t0	19.16	48	2.60	7.20	1.03	29	2.59	71	26.8	67
		t28	5.62	14	0.46	2.68	0.20	21	0.76	79	7.1	18
	8	t0	4.19	52	0.45	0.70	0.17	23	0.6	77	5.5	68
		t28	1.96	24	0.27	0.49	0.10	28	0.3	72	2.7	34
L.v.	8	t0	0.48	6	0.31	0.21	0.05	72	0.02	28	1.7	22
		t28	< lq		< lq	< lq						< lq
	40	t0	2.02	5	1.52	1.23	0.25	75	0.08	25	8.2	21
		t28	0.81	2	0.70	0.56	0.11	78	0.03	22	3.6	9
	200	t0	7.96	4	7.50	8.49	1.22	79	0.32	21	38.6	19
		t28	3.65	2	4.24	4.90	0.69	83	0.15	17	21.0	10

n.a. = not analyzed, lq = limit of quantification, lq for bulk = 0.25 $mg\,kg^{-1}$, lq for water = 0.04 $mg\,l^{-1}$

of 88 %) at the end of the exposure period. The mean analytical findings in bulk sediment were higher than 80% of the nominal spiked concentrations at the start of the exposure period. The small deviation from the 80% rule at the end of the exposure period was tolerated. The arithmetic mean of the measurements at the start and end of the exposure period is 85% of the nominal spiked concentrations. Therefore, the obtained EC_X and NOEC/LOEC values based on nominal concentrations were treated as effective concentrations.

3.2.3.2 *L. variegatus* sediment toxicity test with TBT-Cl

Results of analytical measurements are shown in table 3.11 on page 38. The percentage as a sum of TBT and its metabolites of nominal concentrations found in bulk of 0.8 $mg\,kg^{-1}$ and 100 $mg\,kg^{-1}$ at the start of the exposure period was 84% and 89 % with a mean of 86%. For the lowest tested concentration of 0.0064 $mg\,kg^{-1}$, the measured concentration was higher by a factor of 2.8 than nominal concentrations. This result may only be explained by the fact that this low concentration was close to the limit of quantification. Values of 80% and 103% were found for the 0.8 $mg\,kg^{-1}$ and 100 $mg\,kg^{-1}$ treatment at the end of the exposure period. For the lowest tested concentration of 0.0064 $mg\,kg^{-1}$ the measured concentration was only 52% of nominal concentration. The measurements of the lowest concentrations were excluded due to the uncertainties in the detection. The mean analytical findings in bulk sediment were higher than 80% of the nominal spiked concentrations at the start of the exposure period. Therefore, the obtained EC_X and NOEC/LOEC values based on nominal concentrations were treated as effective concentrations.

3.2.4 Cadmiumchloride

Results of analytical measurements are shown in table 3.12 on page 40.

3.2.4.1 *C. riparius* sediment toxicity test with cadmiumchloride

The percentage of cadmium of nominal concentrations found in bulk at the start of the exposure period ranged from 91% to 94% with a mean of 93% for the 2 and 200 $mg\,kg^{-1}$ treatment. Measured values were below the limit of quantification in the lowest concentration. A mean of 96% (minimum of 94% and a maximum of 98%) was found at the end of the exposure period. The mean analytical findings in bulk sediment were higher than 80% of the nominal spiked concentrations at the start and end of the exposure period. The obtained EC_X and NOEC/LOEC values based on nominal concentrations were treated as effective concentrations.

Table 3.11: Results of analytical measurements of TBT-Cl and metabolites of the different compartments of sediment toxicity tests

spec	nominal mg kg^{-1} TBT-Cl	nominal mg kg^{-1} TBT-Sn	time	bulk DBT	bulk MBT	bulk TBT	bulk TTBT	overlying water DBT	overlying water MBT	overlying water TBT	overlying water TTBT	pore water DBT	pore water MBT	pore water TBT	pore water TTBT	amount in water	amount in sed	balance	% of nominal
				mg kg^{-1} dw				μg l^{-1}				μg l^{-1}				μg	mg	mg kg^{-1}	
C.r.	1	0.36	t0	0.04	< lq	0.78	0.02	0.02	0.19	0.12	0.01	< lq	< lq	< lq	< lq	0.13	0.11	0.85	85
			t28	0.03	< lq	0.58	0.04	0.06	0.59	0.24	0.01	0.14	0.10	0.70	0.04	0.34	0.09	0.66	66
	16	5.84	t0	0.42	< lq	16.6	0.36	0.15	3.05	4.55	0.01	0.12	0.52	3.74	< lq	2.92	2.34	17.38	109
			t28	0.48	< lq	12.4	0.51	0.36	0.92	5.79	0.02	0.63	0.34	11.0	0.14	2.74	1.80	13.37	84
	4	1.46	t0	0.10	< lq	3.06	0.09	0.03	0.47	0.91	0.01	0.10	0.07	1.33	0.05	0.54	0.44	3.25	81
			t28	0.14	< lq	3.36	< lq	0.27	2.13	1.07	0.01	0.25	0.32	1.92	< lq	1.31	0.47	3.51	88
L.v.	0.0064	0.0023	t0	< lq	< lq	0.018	< lq	0.02	0.03	< lq	< lq	< lq	< lq	0.37	< lq	0.01	0.0007	0.018	283
			t28	< lq	< lq	0.0033	< lq	0.02	0.02	0.07	0.01	< lq	0.27	0.28	< lq	0.02	0.0001	0.0038	60
	0.8	0.29	t0	0.01	0.01	0.64	< lq	0.08	0.27	0.96	0.02	0.50	0.57	17.5	0.12	0.27	0.03	0.68	85
			t28	0.02	0.02	0.59	< lq	0.51	0.69	1.94	0.02	0.99	0.82	15.6	0.20	0.56	0.03	0.65	81
	100	36.50	t0	1.03	1.32	85.7	1.01	9.09	92.6	754	1.12	31.7	67.0	2700	7.96	146	3.56	93	93
			t28	1.24	1.42	98.7	1.39	4.75	13.0	326	1.37	28.6	50.5	1613	9.38	60	4.11	104	104

lq = limit of quantification, lq for bulk: lq(TBT, DBT, MBT, TTBT) = 0.0024 mg kg^{-1}, lq for water: lq(TBT, DBT, MBT, TTBT) = 0.012 μg l^{-1}

3.2.4.2 *L. variegatus* sediment toxicity test with cadmiumchloride

The percentage of cadmium of nominal concentrations found in bulk of 1.6 $mg\,kg^{-1}$ and 200 $mg\,kg^{-1}$ at the start of the exposure period was 91 % and 78% with a mean of 84%. Measured concentrations were below the limit of quantification (lq = 0.15 $mg\,kg^{-1}$) for the lowest tested concentration of 0.013 $mg\,kg^{-1}$ at the start and end of the test. A mean of 81% was found for the 1.6 $mg\,kg^{-1}$ and 200 $mg\,kg^{-1}$ treatment at the end of the exposure period. Mean analytical findings in bulk sediment were higher than 80% of the nominal spiked concentrations at the start and end of the exposure period. Therefore, the obtained EC_X and NOEC/LOEC values based on nominal concentrations were treated as effective concentrations.

3.2.5 2,4,6-trinitrotoluene

Results of analytical measurements are shown in table 3.13 on page 41.

3.2.5.1 *C. riparius* sediment toxicity test with TNT

Analytical measurements were below the limit of quantification of 0.5 $mg\,kg^{-1}$ for bulk except for one concentration each at the start and end of the exposure period. The arithmetic mean of measurements in bulk of the start and end of the exposure period was approximately 0.5% of nominal concentrations. Analytical measurements were below 80% of the nominal spiked concentrations at the start of the exposure period. Therefore, calculated nominal EC_X and NOEC/LOEC values were corrected and recalculated. Effective concentrations could not be calculated properly due to little analytical data, as described above. Effective concentrations are roughly lower by a factor of 200 than nominal concentrations.

3.2.5.2 *L. variegatus* sediment toxicity test with TNT

Analytical measurements were only in the highest concentration above the limit of quantification of 0.5 $mg\,kg^{-1}$ for bulk at the start and end of the exposure period. The percentage as a sum of TNT and its metabolites of nominal concentrations found in bulk of the 500 $mg\,kg^{-1}$ treatment at the start of the exposure period was 2.9%. 1.7% of nominal concentration was measured in the 200 $mg\,kg^{-1}$ treatment at the end of the exposure period. Analytical measurements were below 80 % of the nominal spiked concentrations at the start of the exposure period. Therefore, calculated nominal EC_X and NOEC/LOEC values were corrected and recalculated to obtain effective concentrations.

3.2.6 Benzo-[a]-pyrene

Results of analytical measurements are shown in table 3.14 on page 43.

Table 3.12: Results of analytical measurements of cadmium of the different compartments of sediment toxicity tests

species	Nominal CdCl2	Nominal Cd	time	BU	% of nominal	OW	PW	amount	% in water	amount	% in sediment	balance	% of
	$mg\,kg^{-1}$			$mg\,kg^{-1}$	Bulk	$\mu g\,l^{-1}$	$\mu g\,l^{-1}$	in water μg	of total	in sed mg	of total	$mg\,kg^{-1}$	nominal
C.r.	0.02	0.01226	t0	< lq		0.149	25.58	0.3					
			t28	< lq		0.155	20.33	0.3					
	2	1.226	t0	1.12	91	8.15	1412	17.1	10.179	0.15	89.8	1.25	102
			t28	1.15	94	4.73	1353	15.3	8.960	0.16	91.0	1.26	103
	200	122.6	t0	115.09	94	1344	76660	1264	7.523	16	92.5	124	102
			t28	119.83	98	1238	53920	997	5.807	16	94.2	127	104
L.v.	0.013	0.00797	t0	< lq		0.07	3.145	0.02					
			t28	< lq		0.134	214.6	0.7					
	1.6	0.9808	t0	0.89	91	2.86		0.5	1.269	0.04	98.7	0.9	92
			t28	0.79	81	10.5	436.2	3.0	8.640	0.03	91.4	0.9	88
	200	122.6	t0	95.52	78	2500	14260	443	10.385	3.82	89.6	106.6	87
			t28	100.32	82	1994	8444	344	7.904	4.01	92.1	108.9	89

lq = limit of quantification, lq for bulk = 0.03 $mg\,kg^{-1}$, lq for water = 0.001 $mg\,l^{-1}$

Table 3.13: Results of analytical measurements of TNT and metabolites of the different compartments of sediment toxicity tests

species	Nominal mg kg^{-1}	time	BU 2,4,6-TNT	BU 2-A-4,6-DNT mg kg^{-1} dw	BU 4-A-2,6-DNT	BU in % of nom	OW 2,4,6-TNT mg l^{-1}	PW 2,4,6-TNT mg l^{-1}	amount in water mg	% in water of total	amount in sed mg	% in sed of total	balance mg kg^{-1}	% of nominal
C.r.	5.1	t0	< lq	< lq	< lq		< lq	< lq						< lq
		t28	< lq	< lq	< lq		< lq	< lq						< lq
	80	t0	0.62	< lq	< lq	0.78	< lq	< lq					0.6	0.8
		t28	< lq	< lq	< lq		< lq	< lq						< lq
	200	t0	< lq	< lq	0.63	0.32	< lq	< lq			0.09		0.6	0.3
		t28	0.56	< lq	< lq	0.28	< lq	< lq			0.08		0.6	0.3
L.v.	0	t0	< lq	< lq	< lq		< lq	< lq						< lq
		t28	< lq	< lq	< lq		< lq	< lq						< lq
	4	t0	< lq	< lq	< lq		< lq	< lq						< lq
		t28	< lq	< lq	< lq		< lq	< lq						< lq
	500	t0	11.19	1.06	2.26	2.90	3.55	1.03	1.3	69.53	0.6	30.5	24.3	4.9
		t28	7.63	< lq	0.72	1.67	< lq	< lq			0.3		8.3	1.7

sed = sediment,
lq = limit of quantification, lq for bulk: lq(2,4,6-TNT) = 0.5 mg kg^{-1}, lq(2-A-4,6-DNT) = 0.5 mg kg^{-1}, lq(4-A-2,6-DNT) = 0.5 mg kg^{-1},
lq for water: lq(2,4,6-TNT) = 0.004 mg l^{-1}

3.2.6.1 *C. riparius* sediment toxicity test with B(a)P

The percentage of B(a)P of nominal concentrations found in bulk at the start of the exposure period ranged from 69% to 77% with a mean of 73% for the three analyzed concentrations. A mean analytical value of 77% (minimum of 71% and a maximum of 84%) of nominal concentrations was found at the end of the exposure period. The mean analytical findings in bulk sediment were lower than 80% of the nominal spiked concentrations at the start of the exposure period. Therefore, calculated nominal EC_X and NOEC/LOEC values were corrected and recalculated to obtain effective concentrations.

3.2.6.2 *L. variegatus* sediment toxicity test with B(a)P

The percentage of B(a)P of nominal concentrations found in bulk at the start of the exposure period ranged from 64% to 88% with a mean of 80% for the three analyzed concentrations. A mean analytical value of 71% (minimum of 66% and a maximum of 81%) of nominal concentrations was found at the end of the exposure period. The mean analytical findings in bulk sediment were 80% of the nominal spiked concentrations at the start of the exposure period but lower than 80 % of the nominal spiked concentrations at the end of the exposure period. Therefore, nominal EC_X and NOEC/LOEC values were corrected and recalculated to obtain effective concentrations.

3.2.7 4,4-Dichlorodiphenyltrichloroethan

3.2.7.1 *C. riparius* sediment toxicity test with DDT

Results of analytical measurements are shown in table 3.15 on page 44. The percentage of DDT of nominal concentrations found in bulk at the start of the exposure period ranged from 57% to 65% with a mean of 61% for the three analyzed concentrations, whereas findings decreased to a mean of 12 % (minimum of 4% and a maximum of 19%) at the end of the exposure period. The mean analytical findings in bulk sediment were lower than 80% of the nominal spiked concentrations at the start of the exposure period. Therefore, calculated nominal EC_X and NOEC/LOEC values were corrected and recalculated to obtain effective concentrations.

3.2.7.2 *L. variegatus* sediment toxicity test with DDT

Results of analytical measurements are shown in table 3.15 on page 44. The percentage of DDT of nominal concentrations found in bulk at the start of the exposure period ranged from 20% to 99% with a mean of 52% for the three analyzed concentrations, whereas findings

Table 3.14: Results of analytical measurements of B(a)P of the different compartments of sediment toxicity tests

spec	nominal $mg\,kg^{-1}$	time	bulk $mg\,kg^{-1}$ dw	BU in % of nominal	OW $\mu g\,l^{-1}$	PW $\mu g\,l^{-1}$	amount in water μg	% in water of total	amount in sed mg	% in sediment of total	balance $mg\,kg^{-1}$	% of nominal
C.r.	10	t0	7.5	75	0.85	15.5	0.47	0.05	1.01	99.95	7.50	75
		t28	7.5	75	< lq	27.5	0.28	0.03	1.01	99.97	7.51	75
	100	t0	68.6	69	4.67	196.9	3.70	0.04	9.26	99.96	68.59	69
		t28	84.0	84	0.13	177.1	1.82	0.02	11.34	99.98	84.03	84
	1000	t0	767.4	77	7.00	1612	18.71	0.02	104	99.98	768	77
		t28	710.0	71	0.36	153.3	1.67	0.002	95.85	99.998	710	71
L.v.	0.06	t0	0.05	77	< lq	0.80	0.002	0.12	0.0020	99.88	0.05	77
		t28	0.04	67	0.10	0.38	0.016	0.95	0.0017	99.05	0.04	68
	8	t0	5.9	74	1.22	31.1	0.29	0.12	0.24	99.88	5.89	74
		t28	5.3	66	6.38	28.7	1.11	0.52	0.21	99.48	5.34	67
	1000	t0	883.7	88	6.87	571.0	2.81	0.01	35.35	99.99	884	88
		t28	806.0	81	33.42	848.4	7.89	0.02	32.24	99.98	806	81

lq = limit of quantification, lq for bulk = 0.005 $mg\,kg^{-1}$, lq for water = 0.02 $\mu g\,l^{-1}$

Table 3.15: Results of analytical measurements of DDT of the different compartments of sediment toxicity tests

spec	nominal $mg\,kg^{-1}$	time	bulk $mg\,kg^{-1}$ dw	BU in % of nominal	OW $\mu g\,l^{-1}$	PW $\mu g\,l^{-1}$	amount in water μg	% in water of total	amount in sed mg	% in sediment of total	balance $mg\,kg^{-1}$	% of nominal
C.r.	0.3	t0	0.17	57	< lq	< lq			0.02		0.17	57
		t28	0.01	4	< lq	< lq			0.0015		0.01	4
	0.9	t0	0.54	60	< lq	< lq			0.07		0.54	60
		t28	0.11	12	< lq	< lq			0.0148		0.11	12
	8.1	t0	5.28	65	0.28	0.66	0.11	0.016	0.71	99.984	5.28	65
		t28	1.54	19	< lq	0.56	0.01	0.003	0.2081	99.997	1.54	19
L.v.	0.2	t0	0.04	20	< lq	< lq			0.00		0.04	20
		t28	0.03	17	< lq	< lq			0.00		0.03	17
	10	t0	3.72	37	0.17	1.86	0.03	0.022	0.15	99.978	3.72	37
		t28	2.09	21	< lq	0.19	0.0006	0.001	0.08	99.999	2.09	21
	500	t0	494.42	99	2.72	27.82	0.52	0.003	19.78	99.997	494.43	99
		t28	462.67	93	3.80	47.50	0.75	0.004	18.51	99.996	462.69	93

lq = limit of quantification, lq for bulk = 0.0015 $mg\,kg^{-1}$, lq for water = 0.1 $\mu g\,l^{-1}$

decreased to a mean of 43 % (minimum of 17% and a maximum of 92%) at the end of the exposure period. The mean analytical findings in bulk sediment were lower than 80% of the nominal spiked concentrations at the start of the exposure period. Therefore, calculated nominal EC_X and NOEC/LOEC values were corrected and recalculated to obtain effective concentrations.

3.2.8 3,4-Dichloroaniline

Detailed description of analytical measurements can be found in OETKEN *et al.* (2001). Mean measured bulk concentrations were only 1.4 % of nominal concentrations for the tests with *L. variegatus* and *C. riparius*. For *C. riparius* sediment toxicity test, measured bulk concentrations ranged from 0.24 to 6.25% of nominal concentrations with higher concentrations at the start of the exposure. For *L. variegatus* sediment toxicity test, measured bulk concentrations ranged from 0.37 to 5.10% of nominal concentrations. Higher concentrations were measured at the start of the exposure period than at the end of the exposure period. The mean analytical findings in bulk sediment were lower than 80% of the nominal spiked concentrations. Thus, calculated nominal EC_X and NOEC/LOEC values were corrected and recalculated to obtain effective concentrations.

3.2.9 Summary of analytical measurements

Analytical measured chemical concentrations found in bulk in the two sediment toxicity test systems deviated little for TBT and Cadmium with findings of more than 80% of nominal concentrations. Intermediate deviations from nominal concentrations were found for B(a)P (amc[1] = 75%), PCP (65%), and DDT (38%). Large deviations were observed for tests with 2,4-DCP, 3,4-DCA, and TNT with 17%, 1.4%, and 1.3% in bulk of nominal concentrations. Other studies have reported measured chemical concentrations that are substantially lower than nominal concentrations (DEWITT *et al.*, 1989; DOUGLAS *et al.*, 1993; BOESE *et al.*, 1995; HOKE *et al.*, 1995; DAY *et al.*, 1998; OETKEN *et al.*, 2001; STEEVENS *et al.*, 2002). The reasons for such discrepancies include biodegradation, volatilization, solubilization or adsorption to the surfaces of glass containers, formation of crystals, and precipitation out of solution (DAY *et al.*, 1998). These processes are dependent on the test substance and the test system used.

3.3 28-d Sediment toxicity tests - Biological results

The results of the 28-day sediment toxicity tests with the invertebrates are discussed separately for each chemical in the following sections. All effect data and discussions in sections 3.3.1 to 3.3.8 are based on nominal concentrations. Sections 3.3.9 to 3.3.10 are based on nominal and effective concentrations.

[1] overall average measured concentration in percent of nominal concentration

3.3.1 Pentachlorophenol

Following, the results of the sediment toxicity tests with PCP are discussed for each test organism.

3.3.1.1 Sediment toxicity test with *L. variegatus*

The following concentrations were tested in the 28-day sediment toxicity test with *L. variegatus*: 0.05, 0.25, 1.25, 6.25, 31.25 $mg\,kg^{-1}$ PCP. A different sediment was used for this test (test 1). This test was performed within the international ring test research and development project of the German Federal Environmental Agency to validate a sediment toxicity test with the endobenthic aquatic oligochaete *L. variegatus*. An OECD guideline (OECD, 2006) was proposed in 2005. Results are published in a separate report (EGELER *et al.*, 2005) as well. A second test (test 2) with PCP was performed using the coarser sediment as described in section 2.4.1 on page 13 with only one replicate per concentration. Test 2 was performed to compare the results of the two sediments. Following, the results of test 1 are described in detail. During test 1, it was obvious that the worms did not burrow into the sediment of the highest concentration within first 24 to 48 hours as observed in the lower concentrations. After 4 to 6 days worms in the highest concentration died due to lysis. Thus, acute toxicity of water-solved PCP is obvious. The observed burrowing inability in the highest concentration of test 1 was not observed in the same nominal concentration of test 2, where worms burrowed into the sediment, even though 100% mortality was observed in this concentration. This is due to the coarser sediment, which allows worms to burrow easily into the sediment.

3.3.1.1.1 Number of worms The mean number of worms in solvent controls was slightly higher than in controls, but not significantly different (figure 3.5). No worms were found in the highest concentration (31.25 $mg\,kg^{-1}$) after 28 days. This is a significant reduction compared to the pooled controls (p = 0.05, Williams test). In all other treatments, no significant reduction of worm number was observed. There was no clear concentration effect relationship for concentration one to four (0.05 to 6.25 $mg\,kg^{-1}$). For the endpoint total worm number, NOEC/LOEC values of 6.25/31.25 $mg\,kg^{-1}$ were derived. An EC_{10} of 6.2 $mg\,kg^{-1}$ and an EC_{50} of 13.1 $mg\,kg^{-1}$ were calculated using probit analysis. In test 2 (with coarser sediment), an EC_{50} of 17.9 $mg\,kg^{-1}$ was calculated using probit analysis, which is in good agreement with the value obtained in test 1 with the different sediment.

3.3.1.1.2 Biomass There was no significant difference in worm biomass of controls and solvent controls. No worms were found in the highest concentration (31.25 $mg\,kg^{-1}$, figure 3.6). This is a significant reduction compared to the pooled controls (p = 0.05, Williams test). The mean biomass was reduced starting at the lowest concentration 0.05 $mg\,kg^{-1}$ compared to solvent control and control. This reduction was not significant (p = 0.05, Williams test). For

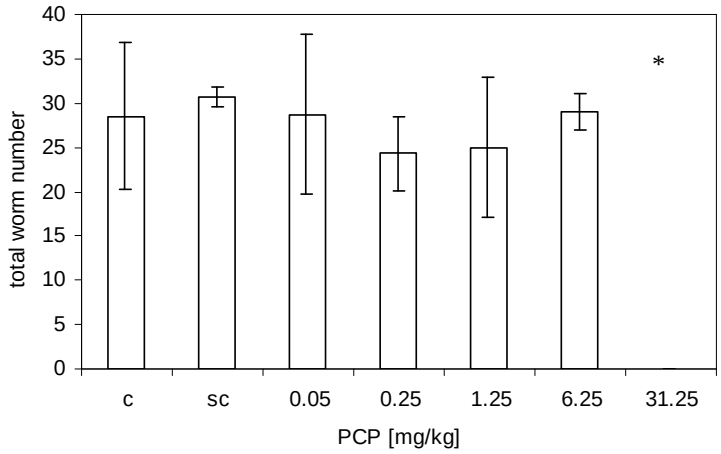


Figure 3.5: Total number of worms after 28 d exposition with PCP, error bars indicate standard deviation, * = significantly different to pooled controls (p = 0.05, Williams test)

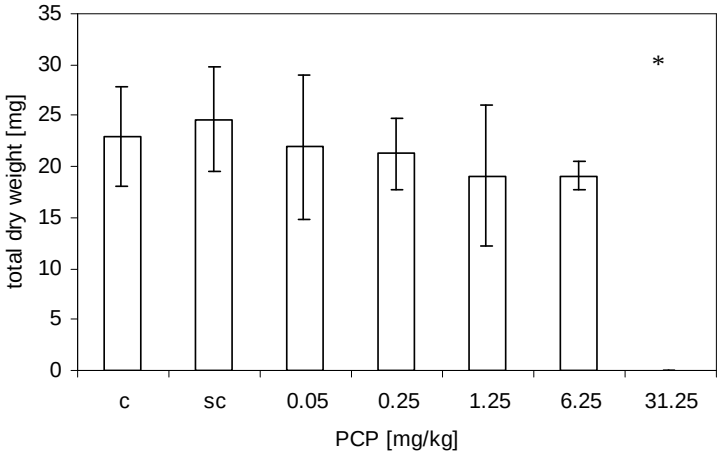


Figure 3.6: Total dry weight of *L. variegatus* after 28 d exposition with PCP, error bars indicate standard deviation, * = significantly different to pooled controls (p = 0.05, Williams test)

the endpoint biomass, NOEC/LOEC values of 6.25/31.25 $mg\,kg^{-1}$ were derived. An EC_{10} of 1.0 $mg\,kg^{-1}$ and an EC_{50} of 7.7 $mg\,kg^{-1}$ were calculated using probit analysis. In test 2, an EC_{50} of 12.9 $mg\,kg^{-1}$ was calculated using probit analysis. This value coincides with the value obtained by test 1 with the different sediment.

3.3.1.1.3 Summary of *L. variegatus* sediment toxicity test with PCP EC_X values with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.16 on page 85 (in $mg\,kg^{-1}$) and in table 3.17 on page 86 (in $\mu mol\,kg^{-1}$). For both endpoints, NOEC/LOEC values of 6.25/31.25 $mg\,kg^{-1}$ were derived. The EC_{50} for the endpoint biomass of 7.7 $mg\,kg^{-1}$ was lower by a factor of 1.7 than for the number of worms. This is due to the relatively larger decrease in biomass compared to the total worm number at the lower concentrations. At the low concentrations the decrease in worm number was not as clear.

EC_{50} values obtained in test 2 were 12.9 / 17.9 mg kg^{-1} for total dry weight / total number of worms. These values coincide with the values in test 1. Sediment composition of the two tested sediments may have no influence on the toxicity of PCP.

3.3.1.2 Sediment toxicity test with *C. riparius*

The following concentrations were tested in the 28-day sediment toxicity test with *C. riparius*: 0.05, 0.5, 5, 50, 500 mg kg^{-1} PCP.

3.3.1.2.1 Emergence Emergence of *C. riparius* after exposure to various concentrations of PCP is shown in figure 3.7. There was no significant difference in total emergence of controls and solvent controls. No midges emerged in the highest concentration of 500 mg kg^{-1}. There was no clear concentration effect relationship from 0.05 to 50 mg kg^{-1}. Emergence of both male and female midges was significantly reduced according to the Williams test (p = 0.05) in the 500 mg kg^{-1} treatment. For the lower concentrations, no significant difference to the pooled controls was observed. NOEC/LOEC values of 50/500 mg kg^{-1} were derived for the endpoint emergence of male and female midges. There was only a 12% decrease in emergence for male midges and no effects for female in the 50 mg kg^{-1} treatment. The next concentration showed 100% effect. It was not meaningful to calculate EC_{10}. EC_{50} of 97.4 mg kg^{-1} was calculated using probit analysis for the endpoint emergence of male midges. For the emergence of female midges the value was 151.9 mg kg^{-1}. Probit analysis was possible but may not be the correct way for EC_{50} calculation for this data set. Therefore, the EC_{50} was also calculated by the geometric mean of 50 and 500 mg kg^{-1}, which resulted in 158 mg kg^{-1}. This value coincides (difference smaller than factor 2) with the calculated EC_{50} values by probit method.

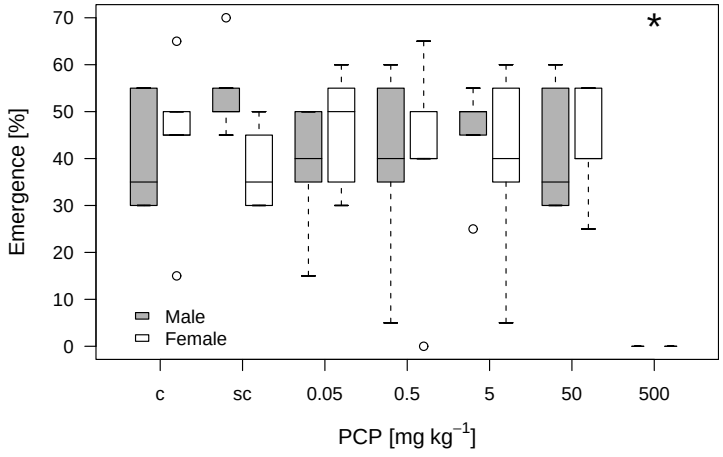


Figure 3.7: Total emergence of *C. riparius* after exposure to various concentrations of PCP, c = control, sc = solvent control, * = significantly different to pooled controls (p = 0.05, Williams test)

Cumulative emergence curves of midges are shown in figure 3.8. A shift in time at which emergence occurs is significant if significant differences are observed for development rates (see following section 3.3.1.2.2). Differences in cumulative emergence curves are visible until day 20. But differences in time shift of the cumulative emergence curves were not significant over the 28-day period, which is supported by observation of no significant effects on development rates.

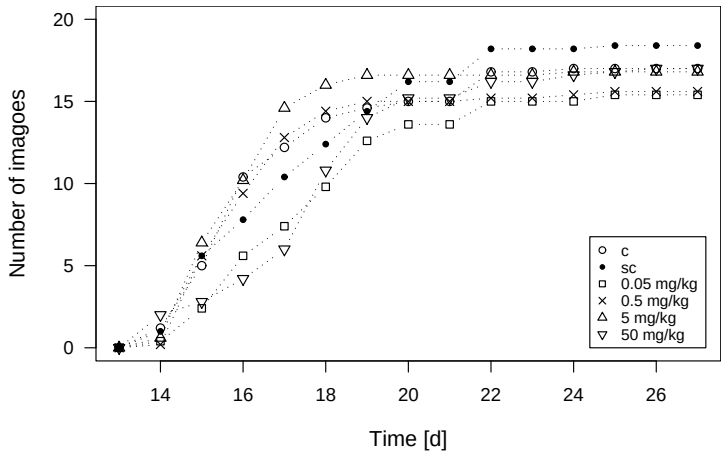


Figure 3.8: Cumulative emergence of *C. riparius* after exposure to various concentrations of PCP, c = control, sc = solvent control, mean of all replicates

3.3.1.2.2 Development rate Development rate of male and female midges is shown in figure 3.9. There was no significant difference in the development rate of controls and solvent controls. No midges emerged in the highest concentration of 500 $mg\,kg^{-1}$. There was no significant difference between the four lowest concentrations and the pooled controls. For the endpoint development rate of both male and female *C. riparius*, NOEC/LOEC values of 50/500 $mg\,kg^{-1}$ were derived.

3.3.1.2.3 Biomass Individual body dry weight of emerged male and female midges is shown in figure 3.10. There was no significant difference in individual and total biomass of controls and solvent controls. Individual body dry weight of both male and female *C. riparius* was significantly reduced in the 5 and 50 $mg\,kg^{-1}$ treatment. For the endpoint individual dry weight of both male and female *C. riparius*, NOEC/LOEC values of 0.5/5 $mg\,kg^{-1}$ were derived.

An EC_{10} of 1.1 $mg\,kg^{-1}$ and an EC_{50} of 45.8 $mg\,kg^{-1}$ were calculated using probit analysis for the endpoint total dry weight of male midges. For the total dry weight of female midges, an EC_{10} of 51.5 and an EC_{50} of 95.8 $mg\,kg^{-1}$ were derived by non-linear regression using the 4-parameter-logistic function. For the endpoint biomass of both male and female *C. riparius*, NOEC/LOEC values of 50/500 $mg\,kg^{-1}$ were derived.

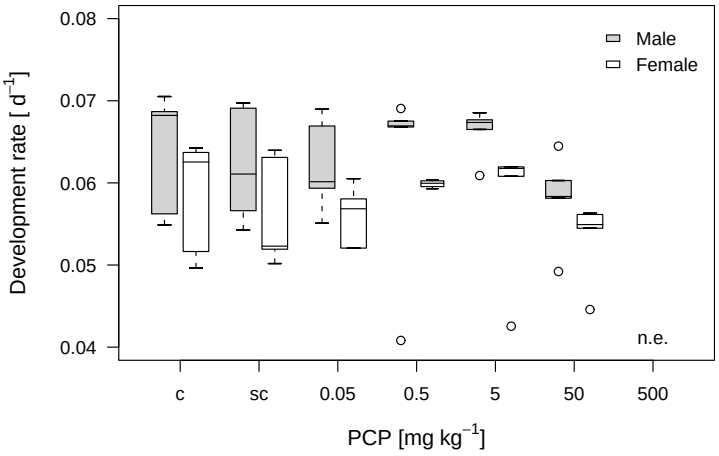


Figure 3.9: Development rate of *C. riparius* after exposure to various concentrations of PCP, c = control, sc = solvent control, n.e. = no emergence

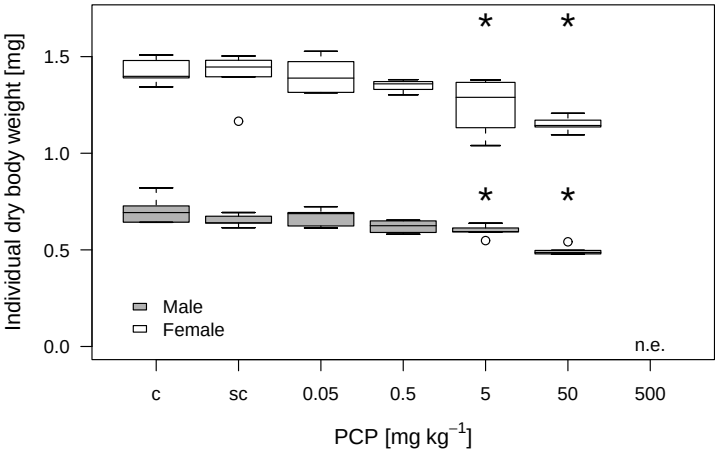


Figure 3.10: Individual body dry weight of male and female imagoes after 28 d exposition with PCP, * = significantly different to solvent control (p = 0.05, Williams test), n.e. = no emergence

3.3.1.2.4 Summary of *C. riparius* sediment toxicity test with PCP EC_X values with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.18 on page 88 (in mg kg^{-1}) and in table 3.19 on page 91 (in μmol kg^{-1}). The lowest NOEC/LOEC values of 0.5/5 mg kg^{-1} were derived for the endpoints individual body dry weight of male and female imagoes. Total emergence was not inhibited up to 50 mg kg^{-1}, but imagoes of the 5 and 50 mg kg^{-1} treatment were significantly lighter than imagoes of pooled controls. EC_{50} of total dry weight of female was lower by a factor of 1.6 than endpoint total emergence of female midges. EC_{50} of total dry weight of males was lower by a factor of 2.1 than endpoint total emergence of male midges.

3.3.1.3 Summary of sediment toxicity tests with PCP

EC_{50} values for *C. riparius* coincide with data for *L. variegatus*. For both organisms the endpoint dry weight was more sensitive than the total number or total emergence.

3.3.2 2,4-Dichlorophenol

Following, the results of the sediment toxicity tests with 2,4-DCP are discussed for each test organism.

3.3.2.1 Sediment toxicity test with *L. variegatus*

The following concentrations were tested in the 28-day sediment toxicity test with *L. variegatus*: 1.6, 8, 40, 200, 1000 $mg\,kg^{-1}$ 2,4-DCP. Only one replicate per concentration was tested.

3.3.2.1.1 Number of worms There was no obvious difference in the number of worms of controls and solvent controls. No worms were found in the highest concentration (1000 $mg\,kg^{-1}$) after 28 days (figure 3.11). Worm numbers were reduced by 75% in the 200 $mg\,kg^{-1}$ treatment compared to the solvent control. There was a clear concentration effect relationship for the three highest concentrations tested (40 to 1000 $mg\,kg^{-1}$). A NOEC/LOEC was estimated according to the method described in section 2.4.4 on page 17. For the endpoint total worm number, NOEC/LOEC values of 40/200 $mg\,kg^{-1}$ were estimated. An EC_{10} of 29.6 $mg\,kg^{-1}$ and an EC_{50} of 102.2 $mg\,kg^{-1}$ were calculated using probit analysis.

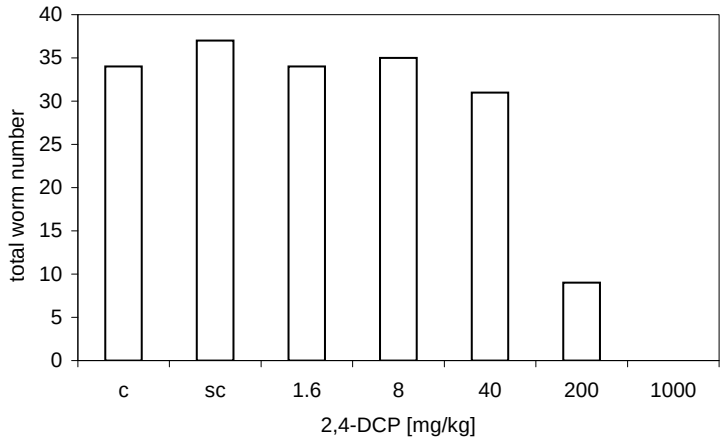


Figure 3.11: Total number of worms after 28 d exposition with DCP, only one replicate per concentration

3.3.2.1.2 Biomass There was no obvious difference in worm biomass of controls and solvent controls. No worms were found in the highest concentration (1000 $mg\,kg^{-1}$, figure 3.12). Worm biomass was reduced by 89% in the 200 $mg\,kg^{-1}$ treatment compared to the solvent control. The biomass also reflects the concentration effect relationship that was observed for the number of worms in the three highest concentrations (40 to 1000 $mg\,kg^{-1}$). For the endpoint biomass, NOEC/LOEC values of 40/200 $mg\,kg^{-1}$ were estimated according to the method described in section 2.4.4 on page 17. An EC_{10} of 70 $mg\,kg^{-1}$ and an EC_{50} of 120.3 $mg\,kg^{-1}$ were calculated using probit analysis.

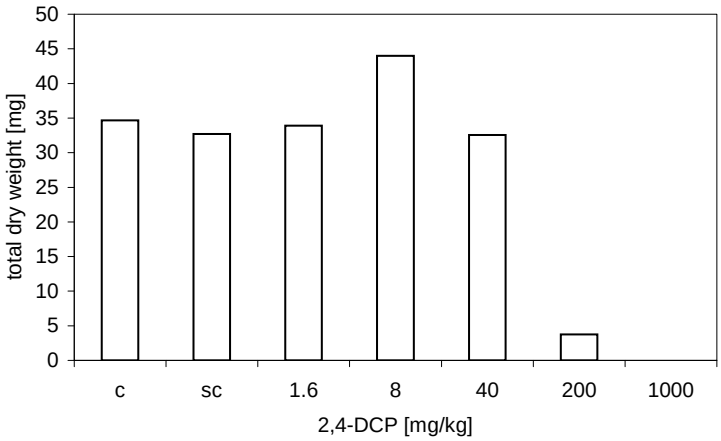


Figure 3.12: Total dry weight of *L. variegatus* after 28 d exposition with DCP, only one replicate per concentration

3.3.2.1.3 Summary of *L. variegatus* sediment toxicity test with 2,4-DCP EC_X values with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.16 on page 85 (in $mg\,kg^{-1}$) and in table 3.17 on page 86 (in $\mu mol\,kg^{-1}$). For both endpoints, NOEC/LOEC values of 40/200 $mg\,kg^{-1}$ were derived. The EC_{50} for the endpoint biomass of 120 $mg\,kg^{-1}$ coincides with the EC_{50} of 102 $mg\,kg^{-1}$ for the endpoint number of worms.

3.3.2.2 Sediment toxicity test with *C. riparius*

The following concentrations were tested in the 28-day sediment toxicity test with *C. riparius*: 8, 17.9, 40, 89, 200 $mg\,kg^{-1}$ 2,4-DCP.

3.3.2.2.1 Emergence Emergence of *C. riparius* after exposure to various concentrations of 2,4-DCP is shown in figure 3.13. No midges emerged in one control replicate. Further, for solvent controls no emergence of male *C. riparius* was observed in one replicate and in another replicate no female midges and only one male midge emerged. For the latter replicate

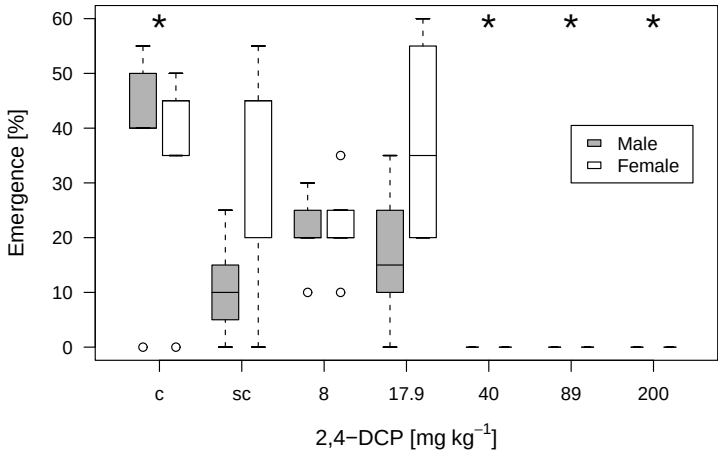


Figure 3.13: Total emergence of *C. riparius* after exposure to various concentrations of 2,4-DCP, c = control, sc = solvent control, * = significantly different to solvent control (p = 0.05, Williams test)

a failure in aeration was the reason. There was no explanation for the failures in emergence of the other replicates. The validity criterion of a mean total emergence of at least 50% in solvent controls was not fulfilled for this test. The mean total emergence was 44% with a median value of 55%. This is due to very small total emergence (sum of male and female) of 5% (one male midge) in one replicate. This result can be explained by a failure in aeration due to technical reasons. Failure in aeration led to a deficit of oxygen, which may have led to the mortality of the larvae. Nevertheless, the test was still considered valid. Controls of this test showed a mean emergence of 72% with a median of 85%. The mean total emergence of male/female *C. riparius* was reduced by 70%/ 6% (for male significantly different, Student-t Test, p = 0.05) in solvent control compared to control. Therefore, treatments were compared with solvent controls. No midges emerged in the 40, 89 and 200 $mg\,kg^{-1}$ treatments. Emergence of both male and female midges was significantly reduced according to the Williams test (p = 0.05) in the 40, 89 and 200 $mg\,kg^{-1}$ treatment, where no midges emerged. NOEC/LOEC of 17.9/40 $mg\,kg^{-1}$ were derived for the endpoint emergence of male and female midges. An EC_{10} of 23.4 $mg\,kg^{-1}$ and an EC_{50} of 26.8 $mg\,kg^{-1}$ were calculated using probit analysis for the endpoint emergence of both male and female midges.

Cumulative emergence curves of midges are shown in figure 3.14. A shift in time at which emergence occurs is significant if significant differences are observed for development rates (see following section 3.3.2.2.2). Midges of solvent controls and the lowest concentration emerged later than in controls and other treatments, which is supported by significantly higher development rates of control male midges compared to male midges of solvent control. There is no explanation for these differences.

3.3.2.2.2 Development rate The development rate of midges after exposure to 2,4-DCP is shown in figure 3.15. Development rates of male midges in solvent controls were signif-

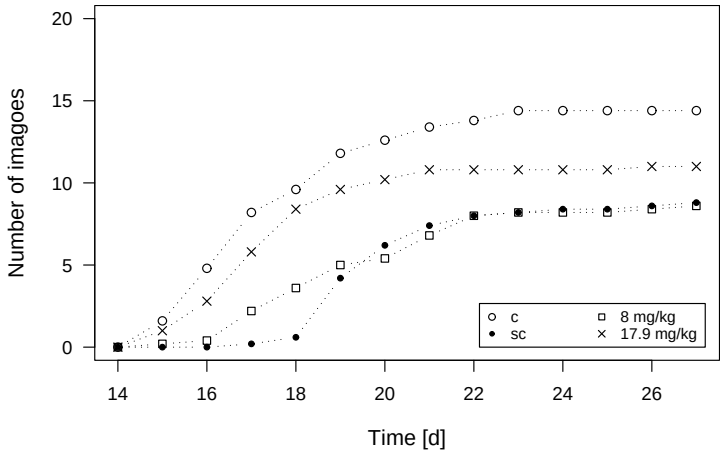


Figure 3.14: Cumulative emergence of *C. riparius* after exposure to various concentrations of 2,4-DCP, c = control, sc = solvent control, mean of all replicates

icantly different from controls (Student-t Test, p = 0.05). Therefore, solvent controls and controls were not pooled. No midges emerged in concentrations 40, 89 and 200 mg kg^{-1}. No significant difference was observed between the development rates of male midges of the two lowest concentrations and the solvent control. For female midges, there was no significant difference between the two lowest concentrations and controls (pooled and separate tested). For the endpoint development rate of both male and female *C. riparius* NOEC/LOEC values of 17.9/40 mg kg^{-1} were derived.

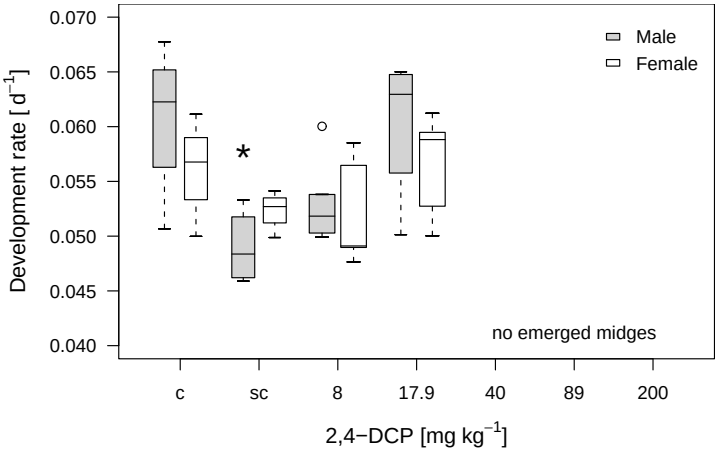


Figure 3.15: Development rate of *C. riparius* after exposure to various concentrations of 2,4-DCP, c = control, sc = solvent control, * = significantly different to control (p = 0.05, Student-t Test)

3.3.2.2.3 Biomass Individual body dry weight of emerged male and female midges is shown in figure 3.16. There was no significant difference in individual and total biomass of controls and solvent controls. Individual body dry weight of both male and female *C. riparius*

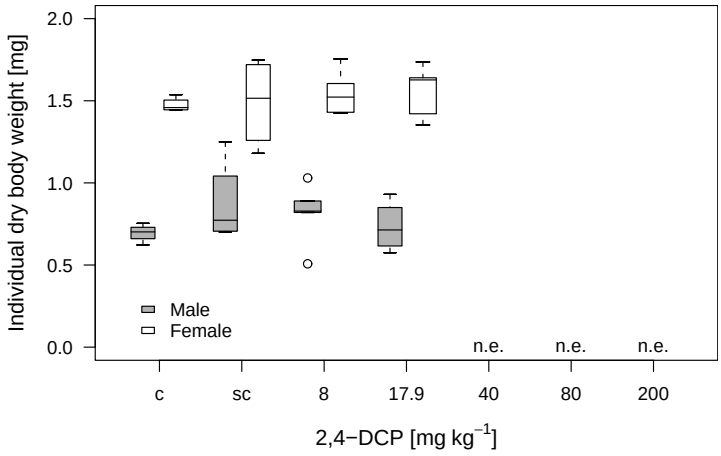


Figure 3.16: Individual body dry weight of male and female imagoes after 28 d exposition with 2,4-DCP, n.e. = no emergence

was significantly reduced in the 40, 80 and 200 mg kg^{-1} treatments, where no midges emerged. For the endpoint individual dry weight of both male and female *C. riparius*, NOEC/LOEC values of 17.9/40 mg kg^{-1} were derived.

An EC_{10} of 23.4 mg kg^{-1} and an EC_{50} of 26.8 mg kg^{-1} were calculated using probit analysis for the endpoint total dry weight of male midges. For the total dry weight of female midges, values were 19.5 and 26.3 mg kg^{-1}, respectively. For the endpoint biomass of both male and female *C. riparius*, NOEC/LOEC values of 17.9/40 mg kg^{-1} were derived.

3.3.2.2.4 Summary of *C. riparius* sediment toxicity test with 2,4-DCP

EC_X values with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.18 on page 88 (in mg kg^{-1}) and in table 3.19 on page 91 (in μmol kg^{-1}). NOEC/LOEC values of 17.9/40 mg kg^{-1} were derived for all observed endpoints. Total emergence as well as total and individual biomass and development rate were not inhibited up to 17.9 mg kg^{-1}.

3.3.2.3 Summary of sediment toxicity tests with 2,4-DCP

C. riparius reacted more sensitively than *L. variegatus* when exposed to 2,4-DCP. An EC_{50} value of 161.3 μmol kg^{-1} (26.3 mg kg^{-1}) was calculated for the endpoint total dry weight of female midges. This is lower by a factor of 4 than the EC_{50} of 627 μmol kg^{-1}(102 mg kg^{-1}) for *L. variegatus*.

3.3.3 3,4-Dichloroaniline

Following, the results of the sediment toxicity tests with 3,4-DCA are discussed for each test organism.

3.3.3.1 Sediment toxicity test with *L. variegatus*

OETKEN *et al.* (2001) performed a 28-day sediment toxicity test with *L. variegatus* with 3,4-DCA using spiked sediment with a very similar sediment composition and a 14-day aging period. The following results are based on the report of OETKEN *et al.* (2001). Five concentrations from 1 to 625 $mg\,kg^{-1}$ with a spacing factor of five were tested.

3.3.3.1.1 Number of worms A NOEC/LOEC based on nominal concentrations of 5/25 $mg\,kg^{-1}$ was found for the endpoint total worm number. Based on measured concentrations, this corresponds to 0.05 (day 0)-0.03(day 28)/0.12 (day 0)-0.09(day 28), respectively. OETKEN *et al.* (2001) concluded that effects in the LOEC were not attributed to pore water concentrations since measured pore water concentrations were lower by a factor of 48 than the determined LC_{50} (96 h). In the highest concentration of 625 $mg\,kg^{-1}$, pore/overlying water concentrations were in the range of LC_{50} (96 h). A mortality of 100% was observed in this concentration. Effects can be attributed to pore water and overlying water concentration. An EC_{10} of 1.4 $mg\,kg^{-1}$ and an EC_{50} of 27 $mg\,kg^{-1}$ were calculated using probit analysis.

3.3.3.1.2 Biomass A NOEC/LOEC based on nominal concentrations of 5/25 $mg\,kg^{-1}$ was found for the endpoint biomass. Based on measured concentrations, this corresponds to 0.05(day 0)-0.03(day 28)/0.12 (day 0)-0.09 (day 28), respectively. Based on nominal concentrations, an EC_{10} of 0.2 $mg\,kg^{-1}$ and an EC_{50} of 26.2 $mg\,kg^{-1}$ were determined.

3.3.3.1.3 Summary of *L. variegatus* sediment toxicity test with 3,4-DCA EC_X values with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.16 on page 85 (in $mg\,kg^{-1}$) and in table 3.17 on page 86 (in $\mu mol\,kg^{-1}$). For both endpoints, NOEC/LOEC values of 5/25 $mg\,kg^{-1}$ were derived for nominal concentrations. This corresponds to NOEC/LOEC values of 0.05(day 0)-0.03(day 28)/0.12 (day 0)-0.09 (day 28), based on measured concentrations. The EC_{50} (based on nominal concentrations) for the endpoint biomass of 26 $mg\,kg^{-1}$ coincides with the EC_{50} of 27 $mg\,kg^{-1}$ for the endpoint number of worms.

3.3.3.2 Sediment toxicity test with *C. riparius*

OETKEN *et al.* (2001) performed a 28-day sediment toxicity test with *C. riparius* with 3,4-DCA using spiked sediment with the same sediment composition and a 14-day aging period. The only difference was that OETKEN *et al.* (2001) used 2 l beakers. The following results are based on the report of OETKEN *et al.* (2001). Five concentrations from 0.064 to 40 $mg\,kg^{-1}$ with a spacing factor of five were tested.

3.3.3.2.1 Emergence There was no significant difference in total emergence of *C. riparius* in all tested concentrations.

3.3.3.2.2 Development rate Midges of all treatments emerged earlier than solvent controls. Calculated EMT_{50} values, which are the reciprocal of the development rate, were significantly lower in all treatments. A LOEC of 0.064 $mg\,kg^{-1}$ was derived. Based on measured concentrations, the LOEC was 0.004 (day 0) - 0.003 (day 28) $mg\,kg^{-1}$.

3.3.3.2.3 Summary of *C. riparius* sediment toxicity test with 3,4-DCA NOEC / LOEC values are summarized in table 3.18 on page 88 (in $mg\,kg^{-1}$) and in table 3.19 on page 91 (in $\mu mol\,kg^{-1}$). There was no significant difference in total emergence of *C. riparius* in all tested concentrations. The lowest NOEC/LOEC values of -/0.064 $mg\,kg^{-1}$ based on nominal concentrations were derived for the endpoint EMT_{50}, which is the reciprocal of the development rate. This corresponds to a LOEC of 0.004 (day 0) - 0.003 (day 28) based on measured concentrations.

3.3.3.3 Summary of sediment toxicity tests with 3,4-DCA

The lowest effect concentrations for the tested invertebrates were derived for *C. riparius*. At the lowest tested concentration, a shift in emergence time was observed. It is not possible to calculate EC_X values for this endpoint. The LOEC of 0.4 $\mu mol\,kg^{-1}$ (0.064 $mg\,kg^{-1}$) was lower by a factor of 405 than the EC_{50} of 162 $\mu mol\,kg^{-1}$ (26 $mg\,kg^{-1}$) for *L. variegatus*. For *L. variegatus*, the endpoint biomass and number of organisms at the end of the exposure period were of equal sensitivity. For *C. riparius*, the total and individual biomass and total emergence were not inhibited up to the highest concentration of 247 $\mu mol\,kg^{-1}$ (40 $mg\,kg^{-1}$). The observed effect on development rate, which results in a shift of emergence time, might not have such an impact on population as a reduction in emergence rate or biomass, with one exception; if male midges emerge later than female midges, copulation may fail. For 3,4-DCA *C. riparius* would be the least sensitive species of the invertebrates when using the similar endpoints biomass and number of individuals and/or emergence rate for interspecies comparison.

3.3.4 2,4,6-Trinitrotoluene

Following, the results of the sediment toxicity tests with TNT are discussed for each test organism.

3.3.4.1 Sediment toxicity test with *L. variegatus*

The following concentrations were tested in the 28-day sediment toxicity test with *L. variegatus*: 0.032, 0.16, 0.8, 4, 20, 100, 500 mg kg^{-1} TNT. Only one replicate per concentration was tested.

3.3.4.1.1 Number of worms There was no obvious difference in the number of worms of controls and solvent controls. No worms were found in the highest concentration (500 mg kg^{-1}) after 28 days (figure 3.17). Worm numbers were reduced by 28% in the 100 mg kg^{-1} treatment compared to the solvent control. A NOEC/LOEC calculation was estimated according to the method described in section 2.4.4 on page 17. There was no clear concentration effect relationship for concentrations from 0.03 to 20 mg kg^{-1}. For the endpoint total worm number, NOEC/LOEC values of 100/500 mg kg^{-1} were estimated. An EC_{10} of 39.9 mg kg^{-1} and an EC_{50} of 137.5 mg kg^{-1} were calculated using probit analysis.

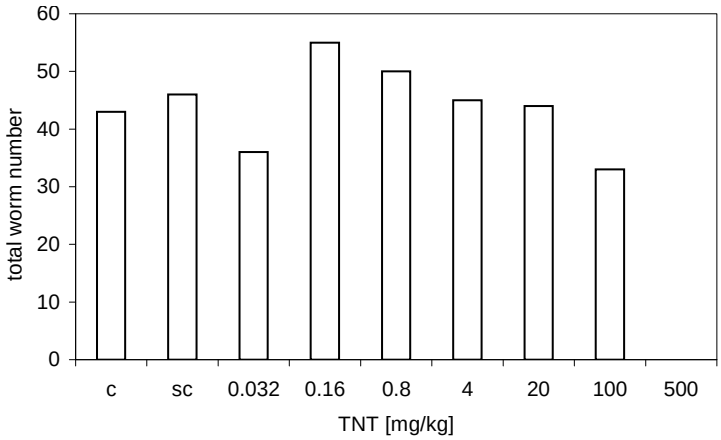


Figure 3.17: Total number of worms after 28 d exposition with TNT, only one replicate per concentration

3.3.4.1.2 Biomass There was no obvious difference in worm biomass of controls and solvent controls. No worms were found in the highest concentration (500 mg kg^{-1}, figure 3.18). There was no clear concentration effect relationship for the endpoint biomass for concentrations 0.03 to 100 mg kg^{-1}. For the endpoint biomass, NOEC/LOEC values of 100/500 mg kg^{-1} were estimated according to the method described in section 2.4.1 on page 13. There was no effect on total worm biomass up to 100 mg kg^{-1}. The next concentration showed 100% effect. It was not meaningful to calculate EC_{10}. EC_{50} of 223.6 mg kg^{-1} was calculated using probit analysis. Probit analysis was possible but may not be the correct way for EC_{50} calculation for this data set. Therefore, the EC_{50} was also calculated by the geometric mean of 100 and 500 mg kg^{-1}, which resulted in 223.6 mg kg^{-1}. This value coincides with the calculated EC_{50} values by probit method using ToxRat® statistical software.

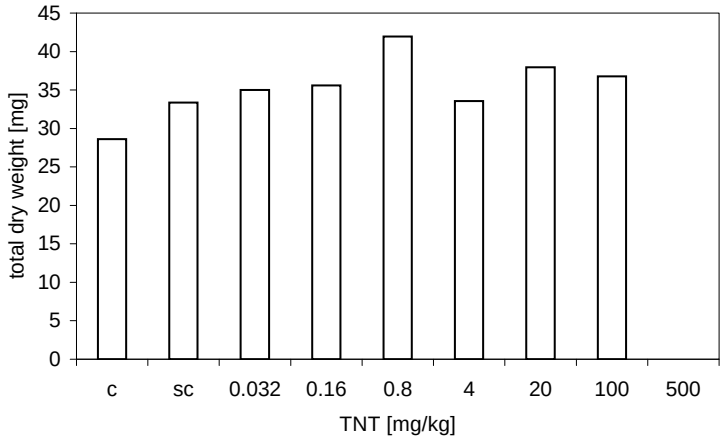


Figure 3.18: Total dry weight of *L. variegatus* after 28 d exposition with TNT, only one replicate per concentration

3.3.4.1.3 Summary of *L. variegatus* sediment toxicity test with TNT EC_X values with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.16 on page 85 (in $mg\,kg^{-1}$) and in table 3.17 on page 86 (in $\mu mol\,kg^{-1}$). For both endpoints, NOEC/LOEC values of 100/500 $mg\,kg^{-1}$ were derived. The EC_{50} for the endpoint number of worms of 138 $mg\,kg^{-1}$ was lower by a factor of 1.6 than for the endpoint biomass. This is due to the relatively lower worm number in the 100 $mg\,kg^{-1}$ treatment. It can be said that EC_{50} values for the endpoint biomass coincide with the worm number. The difference (a factor of 1.6) is very small.

3.3.4.2 Sediment toxicity test with *C. riparius*

The following concentrations were tested in the 28-day sediment toxicity test with *C. riparius*: 5.12, 12.8, 32, 80, 200 $mg\,kg^{-1}$ TNT.

3.3.4.2.1 Emergence Total emergence of male and female *C. riparius* is shown in figure 3.19. Total emergence of *C. riparius* (the sum of male and female midges) was significantly reduced according to the Williams test (p = 0.05) in the 200 $mg\,kg^{-1}$ treatment compared to pooled controls. NOEC/LOEC values of 80/200 were derived for the endpoint total emergence of the sum of male and female midges. An EC_{10} of 98.3 $mg\,kg^{-1}$ and an EC_{50} of 269.9 $mg\,kg^{-1}$ were calculated using probit analysis for the endpoint total emergence.

Cumulative emergence curves of midges are shown in figure 3.20. A shift in time at which emergence occurs is significant if significant differences are observed for development rates (see following section 3.3.4.2.2). Emergence was reduced in the 200 $mg\,kg^{-1}$ treatment and midges emerged later than controls and solvent controls. This delay is supported by the significantly lower development rate of midges exposed to this concentration.

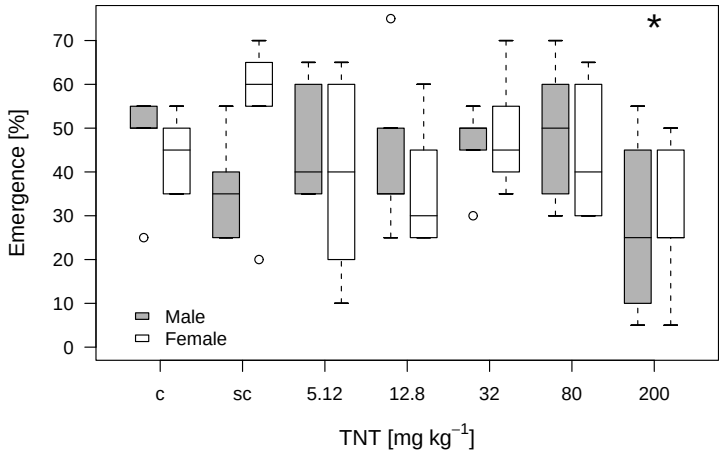


Figure 3.19: Total emergence of *C. riparius* after exposure to various concentrations of TNT, c = control, sc = solvent control, * = significantly different to solvent control (p = 0.05, Williams test)

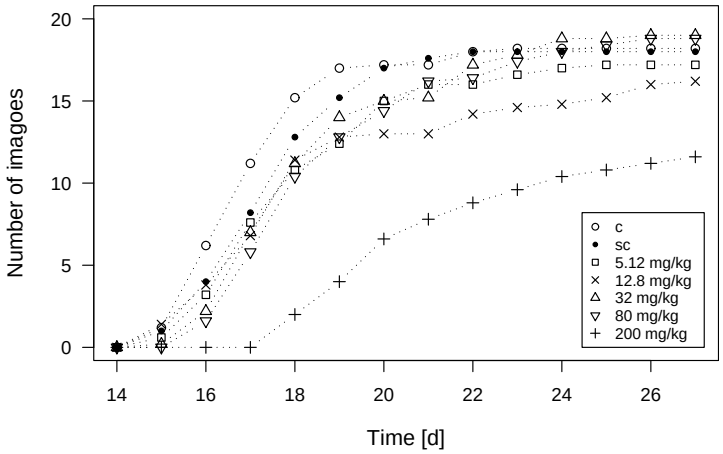


Figure 3.20: Cumulative emergence of *C. riparius* after exposure to various concentrations of TNT, c = control, sc = solvent control, mean of all replicates

3.3.4.2.2 Development rate The development rate of midges after exposure to TNT is shown in figure 3.21. Development rates of male and female midges were significantly reduced compared to pooled controls in the 200 mg kg^{-1} treatment. For the endpoint development rate of both male and female *C. riparius*, NOEC/LOEC values of 80/200 mg kg^{-1} were derived.

3.3.4.2.3 Biomass Individual body dry weight of emerged male and female midges is shown in figure 3.22. Individual body dry weight of both male and female *C. riparius* was not significantly different in any treatments compared to pooled controls. NOEC/LOEC values of 200/- mg kg^{-1} were derived. No significant effects were observed on individual dry weight up to the highest tested concentration.

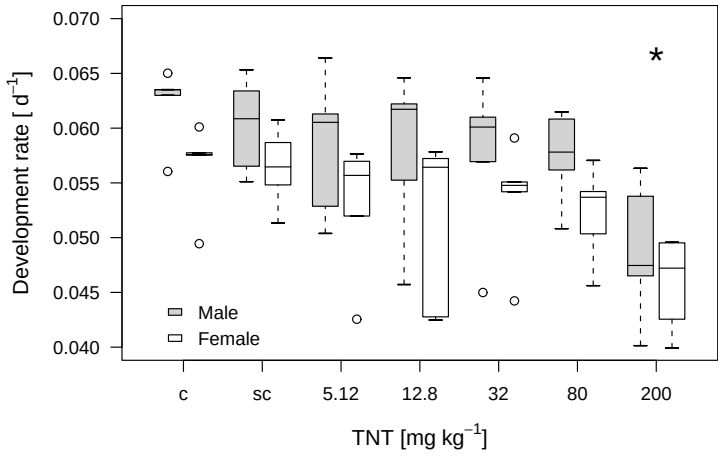


Figure 3.21: Development rate of *C. riparius* after exposure to various concentrations of TNT, c = control, sc = solvent control, * = significantly different to pooled controls (p = 0.05, Williams test)

For the endpoint total biomass of male *C. riparius*, no significant difference was observed. For total biomass of female *C. riparius*, NOEC/LOEC values of 80/200 mg kg^{-1} were derived. An EC_{10} of 6.8 mg kg^{-1} and an EC_{50} of 1170 mg kg^{-1} were calculated using probit analysis for the endpoint total dry weight of female midges. EC_X could not be calculated for male midges due to mathematical reasons.

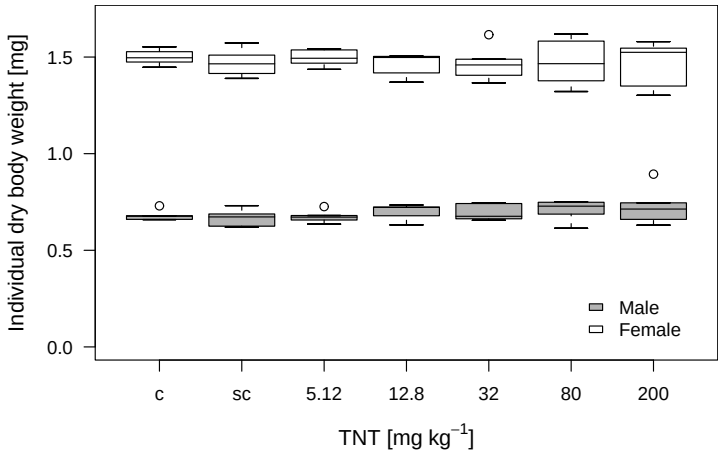


Figure 3.22: Individual body dry weight of male and female imagoes after 28 d exposition with TNT

3.3.4.2.4 Summary of *C. riparius* sediment toxicity test with TNT EC_X values with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.18 on page 88 (in mg kg^{-1}) and in table 3.19 on page 91 (in μmol kg^{-1}). The lowest NOEC/LOEC values of 80/200 mg kg^{-1} were derived for the endpoints development rate of male and female *C. riparius*, total emergence, and total dry weight of female midges. Emergence of male and female midges (when observed separately), total dry weight of male, and

individual dry weight of male and female midges were not inhibited up to the highest tested concentration of 200 $mg\,kg^{-1}$. An EC_{50} value of 270 $mg\,kg^{-1}$ for total emergence was lower by a factor of 4 than the EC_{50} value of 1170 $mg\,kg^{-1}$ for total dry weight of female midges. EC_X calculations were not possible for development rates and the endpoints with no inhibition up to the highest tested concentration. The endpoint total emergence turned out to be the most sensitive endpoint in the sediment toxicity test with TNT.

3.3.4.3 Summary of sediment toxicity tests with TNT

The lowest EC_{50} values for the tested invertebrates were derived for *L. variegatus* with 605 $\mu mol\,kg^{-1}$ (138 $mg\,kg^{-1}$). The lowest EC_{50} of 1188 $\mu mol\,kg^{-1}$ (270 $mg\,kg^{-1}$) for *C. riparius* was higher by a factor of 2. For *L. variegatus*, the endpoint number of organisms found at the end of the exposure period was more sensitive than biomass. Similarities were observed for *C. riparius* for which the endpoints total emergence and development rate were more sensitive than biomass. Differences in invertebrates sensitivity are smaller than a factor of 5, which is considered a very small difference (see detailed description for definitions of differences in species sensitivity in section 3.3.10 on page 93).

3.3.5 4,4-Dichlorodiphenyltrichloroethan

Following, the results of the sediment toxicity tests with DDT are discussed for each test organism.

3.3.5.1 Sediment toxicity test with *L. variegatus*

The following concentrations were tested in the 28-day sediment toxicity test with *L. variegatus*: 0.2, 1.41, 10, 70.71, 500 $mg\,kg^{-1}$ DDT.

3.3.5.1.1 Number of worms Worm numbers were significantly lower by 33% in solvent controls compared to controls (Student-t Test, p = 0.05, figure 3.23). Therefore, treatments were compared with solvent controls. An stimulation of reproduction at the two lowest concentrations with following inhibition at high concentrations was observed, which is typical for so called hormesis. Worm numbers were significantly reduced by 45% and 79% in the 70.7 and 500 $mg\,kg^{-1}$ treatment compared to the solvent control. There was a clear concentration effect relationship from concentration 1.4 to 500 $mg\,kg^{-1}$. For the endpoint total worm number NOEC/LOEC values of 10/70.7 $mg\,kg^{-1}$ were derived. An EC_{10} of 10.1 $mg\,kg^{-1}$ and an EC_{50} of 103 $mg\,kg^{-1}$ were calculated using probit analysis. The hormesis effect is not clear when comparing treatments with controls. The LOEC would then be 10 $mg\,kg^{-1}$. Therefore, this test would be a candidate to repeat but was not done for time and cost saving reasons.

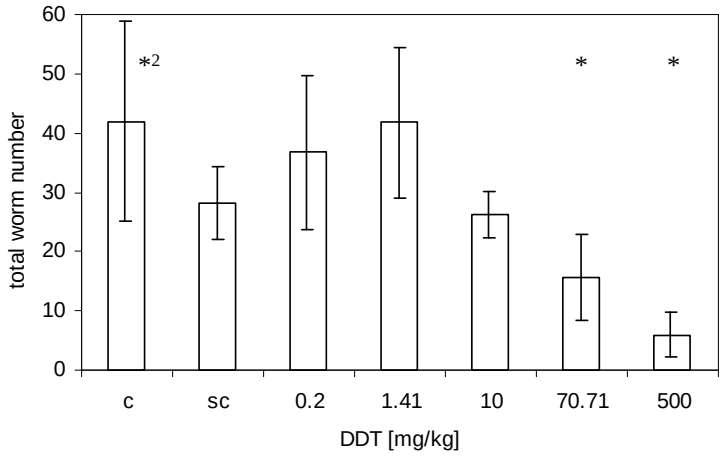


Figure 3.23: Total number of worms after 28 d exposition with DDT, error bars indicate standard deviation, * = significantly different to solvent controls (p = 0.05, Williams test), *2 = significantly different to solvent control (p = 0.05, Student-t Test)

3.3.5.1.2 Biomass Biomass as total dry weight was significantly lower by 42% in solvent controls compared to controls (Student-t Test, p = 0.05, figure 3.24). As for the total number of worms hormesis can be observed for the biomass when comparing to solvent controls. Biomass increased at two lowest concentrations and was significantly reduced by 88% in the 500 mg kg^{-1} treatment compared to the solvent control. There was a clear concentration effect relationship from concentration 1.4 to 500 mg kg^{-1}. For the endpoint total worm number, NOEC/LOEC values of 70.7/500 mg kg^{-1} were derived. An EC_{10} of 159.6 mg kg^{-1} and an EC_{50} of 300.5 mg kg^{-1} were calculated using probit analysis.

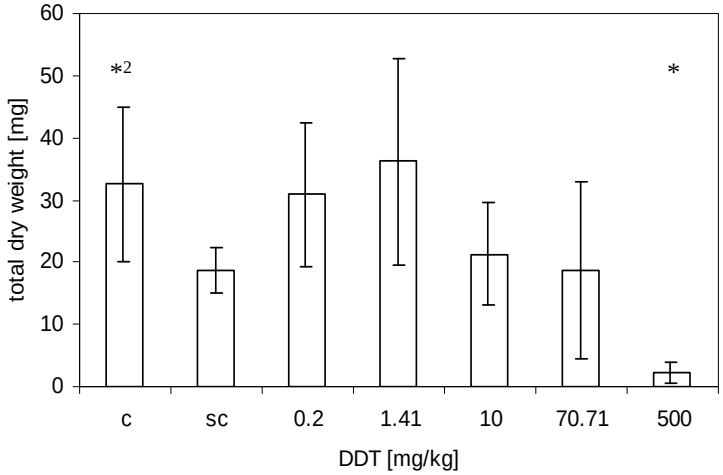


Figure 3.24: Total dry weight of *L. variegatus* after 28 d exposition with DDT, error bars indicate standard deviation, * = significantly different to solvent controls (p = 0.05, Williams test), *2 = significantly different to solvent control (p = 0.05, Student-t Test)

3.3.5.1.3 Summary of *L. variegatus* sediment toxicity test with DDT EC_X values with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.16 on page 85 (in $mg\,kg^{-1}$) and in table 3.17 on page 86 (in $\mu mol\,kg^{-1}$). The lowest NOEC/LOEC values of 10/71 $mg\,kg^{-1}$ were derived for the endpoint total number of worms. The EC_{50} for the endpoint number of worms of 103 $mg\,kg^{-1}$ was lower by a factor of 3 than for the endpoint biomass. This is due to the relatively lower worm number in the 71 $mg\,kg^{-1}$ treatment. The endpoint worm number reacted more sensitively than endpoint biomass in the sediment toxicity test with DDT.

3.3.5.2 Sediment toxicity test with *C. riparius*

The following concentrations were tested in the 28-day sediment toxicity test with *C. riparius*: 0.1, 0.3, 0.9, 2.7, 8.1 $mg\,kg^{-1}$ DDT.

3.3.5.2.1 Emergence Emergence of *C. riparius* after exposure to various concentrations of DDT is shown in figure 3.25. Mortality of 100% percent was observed in one control replicate. This mortality can be explained by failure in aeration due to technical problems. The

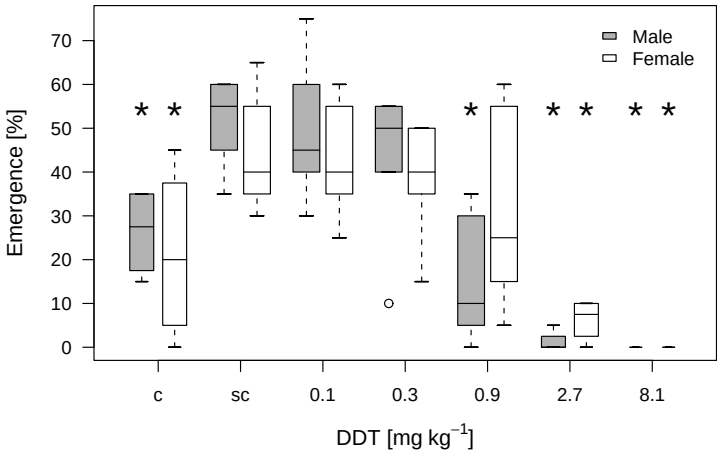


Figure 3.25: Total emergence of *C. riparius* after exposure to various concentrations of DDT, c = control, sc = solvent control, * = significantly different to solvent control (p = 0.05, Williams test)

result was a lack of oxygen causing death to larvaes. Therefore, this replicate was excluded for the calculation. Further, no female midges emerged in another control replicate. There is no explanation for this. Total emergence was reduced by 60% compared to solvent control. Therefore, for all endpoints, treatments were compared with solvent controls. There was a clear concentration effect relationship from the lowest to the highest concentration. No midges emerged in the highest concentration (8.1 $mg\,kg^{-1}$). Total emergence was reduced by 94% in the 2.7 $mg\,kg^{-1}$ treatment, followed by 50%, 17%, and 3% in the 0.9, 0.3, and 0.1 $mg\,kg^{-1}$

treatment, respectively. Emergence of male midges was significantly reduced according to the Williams test (p = 0.05) in the 0.9, 2.7, and 8.1 $mg\,kg^{-1}$ treatment. Emergence of female midges was significantly reduced in the 2.7 and 8.1 $mg\,kg^{-1}$ treatment (p = 0.05, Williams test). A NOEC/LOEC of 0.3/0.9 $mg\,kg^{-1}$ was derived for the endpoint emergence of male midges and 0.9/2.7 $mg\,kg^{-1}$ for the emergence of female midges. An EC_{10} of 0.2 $mg\,kg^{-1}$ and an EC_{50} of 0.6 $mg\,kg^{-1}$ were calculated using probit analysis for the endpoint emergence of male midges. For the emergence of female midges, values were 0.5 and 1.2 $mg\,kg^{-1}$, respectively.

Cumulative emergence curves of midges are shown in figure 3.26. Low emergence was observed in the controls and 0.9 $mg\,kg^{-1}$ treatment. Fairly small emergence was observed in the 2.7 $mg\,kg^{-1}$ treatment. No significant difference was observed in the time at which emergence occurs since no significant differences were observed for development rates, which are described in the following section 3.3.5.2.2.

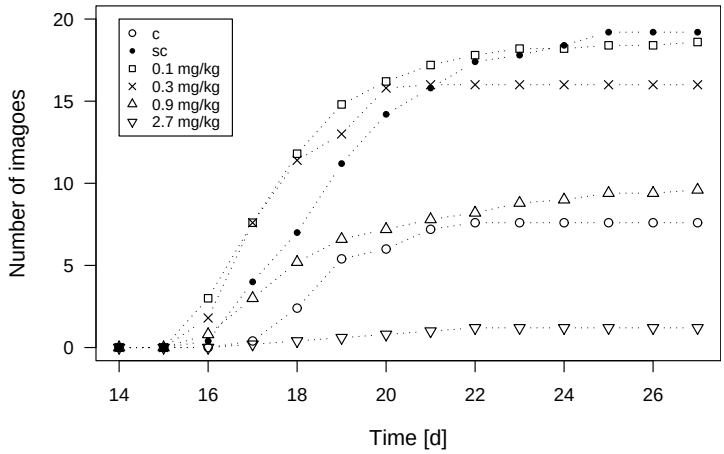


Figure 3.26: Cumulative emergence of *C. riparius* after exposure to various concentrations of DDT, c = control, sc = solvent control, mean of all replicates

3.3.5.2.2 Development rate The development rate of midges is shown in figure 3.27. No midges emerged in the highest concentration of 8.1 $mg\,kg^{-1}$. There is no significant difference between the four lowest concentrations and the pooled controls. For the endpoint development rate of both male and female *C. riparius*, NOEC/LOEC values of 2.7/8.1 $mg\,kg^{-1}$ were derived.

3.3.5.2.3 Biomass Individual body dry weight of emerged male and female midges is shown in figure 3.28. Individual body dry weight was significantly reduced, for the male *C. riparius* in the 0.9 $mg\,kg^{-1}$ treatment and for the female in the 2.7 $mg\,kg^{-1}$ treatment. An EC_{10} of 0.14 $mg\,kg^{-1}$ and an EC_{50} of 0.48 $mg\,kg^{-1}$ were calculated using probit analysis for the endpoint total dry weight of male midges. For the total dry weight of female midges,

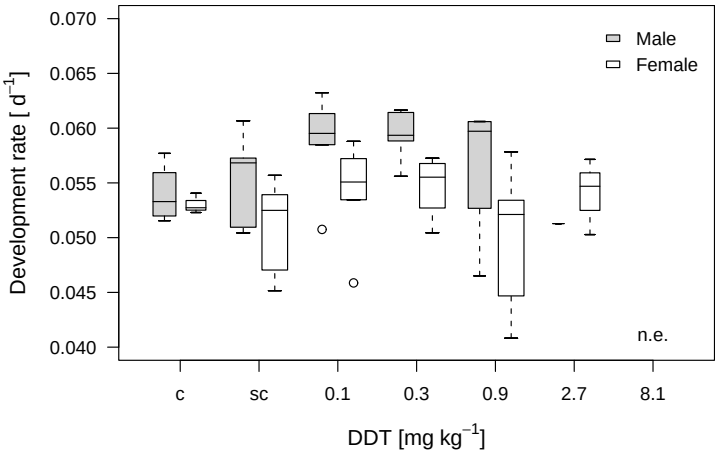


Figure 3.27: Development rate of *C. riparius* after exposure to various concentrations of DDT, c = control, sc = solvent control, n.e. = no emergence

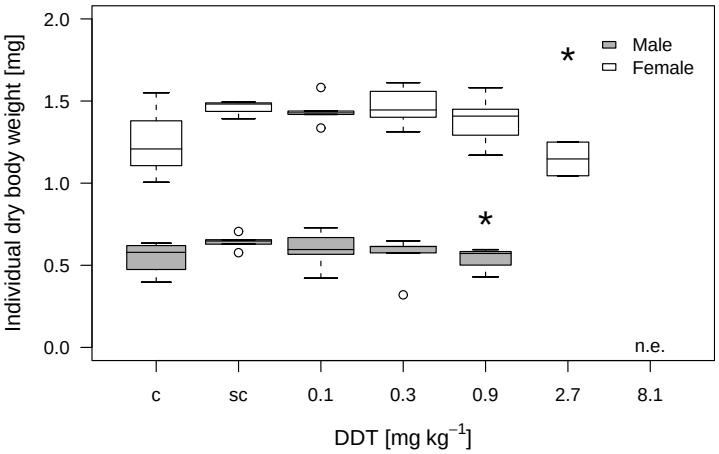


Figure 3.28: Individual body dry weight of male and female imagoes after 28 d exposition with DDT, * = significantly different to solvent control (p = 0.05, Williams test), n.e. = no emergence

values were 0.43 and 1.21 mg kg^{-1}, respectively. For the endpoint biomass of male *C. riparius*, NOEC/LOEC values of 0.3/0.9 mg kg^{-1} were derived and for female *C. riparius*, NOEC/LOEC values of 0.9/2.7 mg kg^{-1} were derived.

3.3.5.2.4 Summary of *C. riparius* sediment toxicity test with DDT EC_X values with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.18 on page 88 (in mg kg^{-1}) and in table 3.19 on page 91 (in μmol kg^{-1}). The lowest NOEC/LOEC values of 0.3/0.9 mg kg^{-1} were derived for the endpoints total emergence, emergence of male midges, and individual and total dry weight of male *C. riparius*. Development rate was not inhibited up to 8.1 mg kg^{-1}. The lowest EC_{50} value of 0.48 mg kg^{-1} was calculated for the total dry weight of male midges. This was lower by a factor of 2.6 than the EC_{50} value of 1.24 mg kg^{-1} for emergence of female midges. EC_X calculation was not possible for

development rates. The endpoint total dry weight of male midges turned out to be the most sensitive endpoint in the sediment toxicity test with DDT. Male midges were more sensitive than female midges, which may result in a disadvantage for the population.

3.3.5.3 Summary of sediment toxicity tests with DDT

C. riparius was the most sensitive of the two invertebrates in the sediment toxicity test with DDT. The lowest EC_{50} value of 1.34 μmol kg^{-1} (0.48 mg kg^{-1}) for *C. riparius* was lower by a factor of 216 than for *L. variegatus*. For *L. variegatus*, biomass was more sensitive than the number of organisms. For *C. riparius*, total emergence and biomass were more sensitive than the development rate. Differences in invertebrates sensitivity are higher than a factor of 10, which is considered a large difference (see detailed description for definitions of differences in species sensitivity in section 3.3.10 on page 93).

3.3.6 Tributyltinchloride

Following, the results of the sediment toxicity tests with TBT-Cl are discussed for each test organism.

3.3.6.1 Sediment toxicity test with *L. variegatus*

The following concentrations were tested in the 28-day sediment toxicity test with *L. variegatus* and TBT-Cl: 0.002, 0.012, 0.058, 0.29, 1.5, 7.3, 36.5 mg kg^{-1} TBT-Sn. Only one replicate per concentration was tested.

3.3.6.1.1 Number of worms Number of worms in control and solvent controls were low compared to the number of worms found in the lowest two concentrations. But numbers are still within the range found for other tests with more than one replicate. A detailed overview on variation for this endpoint is given in table 3.24 on page 125. Hormesis is not discussed, due to only one replicate that was used for biological effects of each concentration and of high variation that was observed for *L. variegatus* test system. No worms were found in the 7.3 and 36.5 mg kg^{-1} TBT-Sn treatment after 28 days (figure 3.29). Worm numbers were reduced by 78% in the 1.5 mg kg^{-1} treatment compared to the solvent control. There was a clear concentration effect relationship from concentration 0.012 to 36.5 mg kg^{-1}. For the endpoint total worm number, NOEC/LOEC values of 0.29/1.5 mg kg^{-1} were estimated according to the method described in section 2.4.4 on page 17. An EC_{10} of 0.72 mg kg^{-1} and an EC_{50} of 1.12 mg kg^{-1} were calculated using probit analysis.

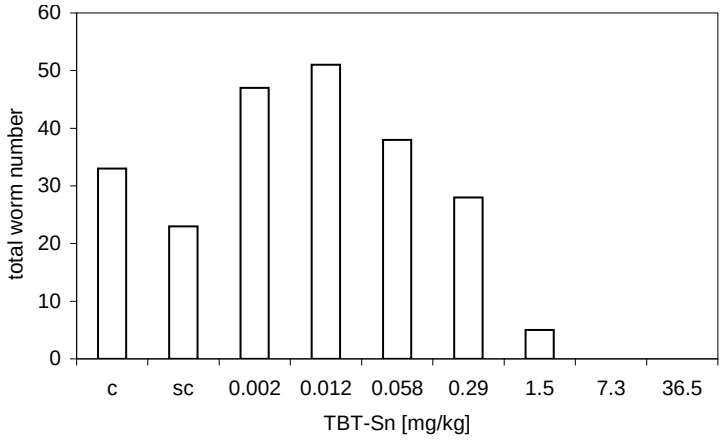


Figure 3.29: Total number of worms after 28 d exposition with TBT-Cl, concentrations refer to TBT-Sn, only one replicate per concentration

3.3.6.1.2 Biomass The low number of worms in controls and solvent controls are reflected in relatively low biomass as well. The rationale from above applies to biomass as well. No worms were found in the 7.3 and 36.5 $mg\,kg^{-1}$ TBT-Sn treatment after 28 days (figure 3.30). Worm biomass was reduced by 90% in the 1.5 $mg\,kg^{-1}$ TBT-Sn treatment compared to

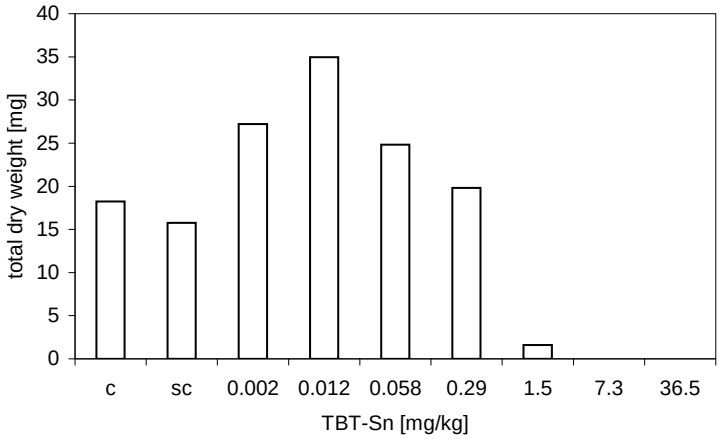


Figure 3.30: Total dry weight of *L. variegatus* after 28 d exposition with TBT-Cl, concentrations refer to TBT-Sn, only one replicate per concentration

the solvent control. The biomass also reflects the concentration effect relationship that was observed for the total worm number from concentration 0.01 to 36.5 $mg\,kg^{-1}$. For the endpoint biomass, NOEC/LOEC values of 0.29/1.5 $mg\,kg^{-1}$ were estimated according to the method described in section 2.4.4 on page 17. An EC_{10} of 0.66 $mg\,kg^{-1}$ and an EC_{50} of 0.98 $mg\,kg^{-1}$ were calculated using probit analysis.

3.3.6.1.3 Summary of *L. variegatus* sediment toxicity test with TBT-Cl EC_X values with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in

table 3.16 on page 85 (in $mg\,kg^{-1}$) and in table 3.17 on page 86 (in $\mu mol\,kg^{-1}$). For both endpoints total worm number and biomass NOEC/LOEC values of 0.3/1.5 $mg\,kg^{-1}$ TBT-Sn were derived. The EC_{50} of 1.12 $mg\,kg^{-1}$ TBT-Sn for the endpoint number of worms coincided with the EC_{50} of 0.98 $mg\,kg^{-1}$ TBT-Sn for the endpoint biomass. Both endpoints exhibited nearly the same sensitivity in the sediment toxicity test with TBT-Cl.

3.3.6.2 Sediment toxicity test with *C. riparius*

The following concentrations were tested in the 28-day sediment toxicity test with *C. riparius* and TBT-Cl: 0.37, 0.7, 1.5, 2.9, 5.8 $mg\,kg^{-1}$ TBT-Sn.

3.3.6.2.1 Emergence Total emergence of *C. riparius* after exposure to various concentrations of TBT-Cl is shown in figure 3.31. Total emergence of *C. riparius* was reduced by

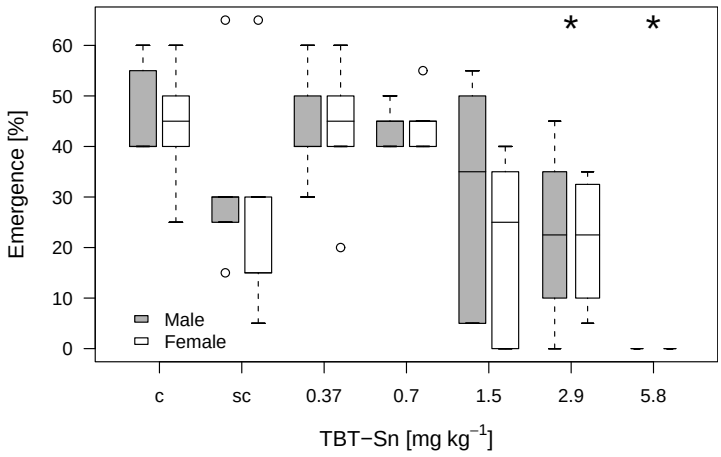


Figure 3.31: Total emergence of *C. riparius* after exposure to various concentrations of TBT-Cl, c = control, sc = solvent control, * = significantly different to pooled control (p = 0.05, Williams test)

35% (but not significantly) in solvent control compared to control. Therefore, treatments were compared with pooled controls and solvent controls. There was no explanation for this observation. With a mean emergence of 59% it was the second lowest of all tests (see table 3.30 on page 130). There was a clear concentration effect relationship from 0.7 to 5.8 $mg\,kg^{-1}$ TBT-Sn. No midges emerged in the highest concentration (5.8 $mg\,kg^{-1}$ TBT-Sn). Total emergence was reduced by 42% in the 2.9 $mg\,kg^{-1}$ treatment followed by 33% in the 1.5 $mg\,kg^{-1}$ treatment. Emergence of male and female midges was significantly reduced according to the Williams test (p = 0.05) in the 2.9 and 5.8 $mg\,kg^{-1}$ treatment. A NOEC/LOEC of 1.5/2.9 were derived for the endpoint emergence of male and female midges. An EC_{10} of 1.1 $mg\,kg^{-1}$ and an EC_{50} of 2.4 $mg\,kg^{-1}$ were calculated using probit analysis for the endpoint emergence of male midges. For the emergence of female midges, values were 0.8 and 2.2 $mg\,kg^{-1}$, respectively.

Cumulative emergence curves of midges are shown in figure 3.32. A shift in time at which emergence occurs is significant if significant differences are observed for development rates (see following section 3.3.6.2.2). Compared to controls, less and later emergence was observed in the solvent controls compared to controls. This time shift in emergence between sol-

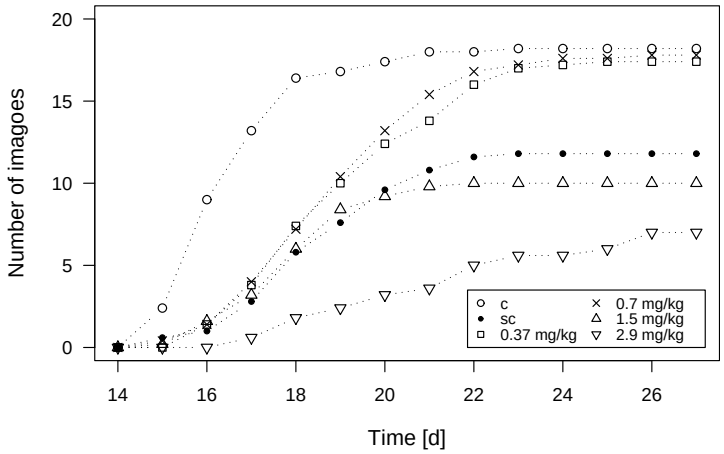


Figure 3.32: Cumulative emergence of *C. riparius* after exposure to various concentrations of TBT-Cl, no emergence in the highest concentration

vent controls and controls cannot be explained. Fairly small and late emergence was observed in the 2.9 $mg\,kg^{-1}$ treatment. The later emergence agree with the significantly differences in the calculated development rates for solvent controls and treatment 2.9 $mg\,kg^{-1}$.

3.3.6.2.2 Development rate The development rate of midges is shown in figure 3.33. No midges emerged in the highest concentration tested. Development rates of solvent controls were significantly different than controls (Student-t Test, p = 0.05). This earlier emergence of control midges cannot be explained. Therefore, treatments were compared with solvent controls. No significant difference was observed between the development rates of male midges of the four lowest concentrations and the solvent control. Development rate of female midges of the 2.9 $mg\,kg^{-1}$ treatment was significantly lower than in solvent controls. NOEC/LOEC values for the development rate of female midges of 1.5/2.9 $mg\,kg^{-1}$ were derived.

3.3.6.2.3 Biomass Individual body dry weight of emerged male and female midges is shown in figure 3.34. Individual body dry weight of both male and female *C. riparius* was significantly reduced in the 2.9 $mg\,kg^{-1}$ treatment. An EC_{10} of 0.9 $mg\,kg^{-1}$ and an EC_{50} of 2.0 $mg\,kg^{-1}$ were calculated using probit analysis for the endpoint total dry weight of male midges. For the total dry weight of female midges, values were 0.8 and 1.9 $mg\,kg^{-1}$, respectively. For the endpoint biomass of both male and female *C. riparius*, NOEC/LOEC values of 1.5/2.9 $mg\,kg^{-1}$ were derived.

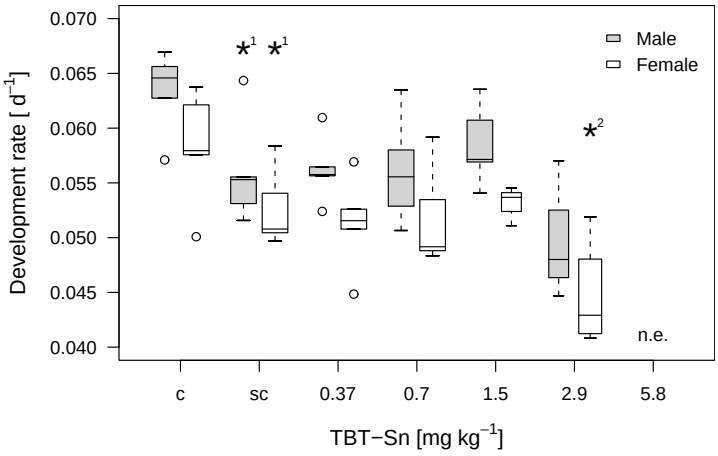


Figure 3.33: Development rate of *C. riparius* after exposure to various concentrations of TBT-Cl, c = control, sc = solvent control, n.e. = no emergence, $*^1$ = significantly different to control (p = 0.05, Student-t Test), $*^2$ = significantly different to solvent control (p = 0.05, Williams test)

3.3.6.2.4 Summary of *C. riparius* sediment toxicity test with TBT-Cl

EC_X values with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.18 on page 88 (in $mg\,kg^{-1}$) and in table 3.19 on page 91 (in $\mu mol\,kg^{-1}$). The lowest NOEC/LOEC values of 1.46/2.92 $mg\,kg^{-1}$ TBT-Sn were derived for all endpoints with the exception of the development rate. The development rate of male and female *C. riparius* was not inhibited up to 2.92 $mg\,kg^{-1}$ TBT-Sn. EC_{50} values ranging from 1.9 $mg\,kg^{-1}$ TBT-Sn for the endpoint total dry weight of female midges to 2.45 $mg\,kg^{-1}$ TBT-Sn for the endpoint emergence of male midges coincided with each other. EC_X calculation was not possible for development rates. Nonetheless the endpoint total dry weight of female and male midges turned out to be the most sensitive endpoint in the sediment toxicity test with TBT-Cl.

3.3.6.3 Summary of sediment toxicity tests with TBT-Cl

L. variegatus was the most sensitive of the two invertebrates in the sediment toxicity test with TBT-Cl. The following concentrations are referred to TBT-Sn. The lowest EC_{50} value of 3 $\mu mol\,kg^{-1}$ (1 $mg\,kg^{-1}$) for *L. variegatus* was lower a by factor of 2 than for *C. riparius* (1.9 $mg\,kg^{-1}$). For *L. variegatus*, individual endpoints were of equal sensitivity, whereas for *C. riparius* only the development rate of male midges was inhibited at higher concentration than the other endpoints. Differences in invertebrates sensitivity are lower than factor of 5, which is considered a very small difference (see detailed description for definitions of differences in species sensitivity in section 3.3.10 on page 93).

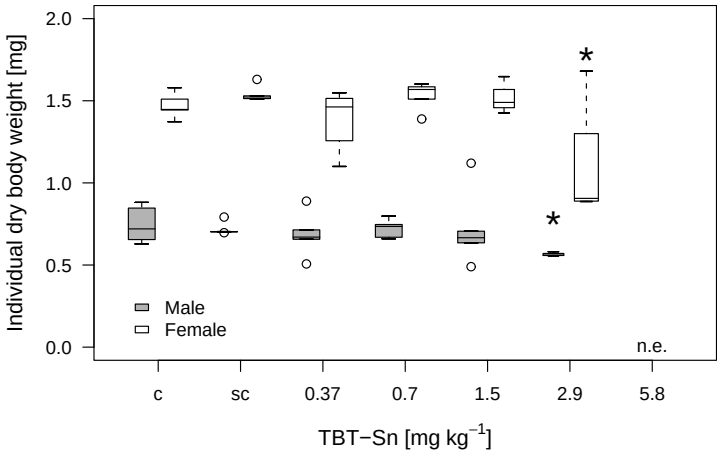


Figure 3.34: Individual body dry weight of male and female imagoes after 28 d exposition with TBT-Cl, * = significantly different to solvent control (p = 0.05, Williams test), n.e. = no emergence

3.3.7 Cadmiumchloride

Following, the results of the sediment toxicity tests with cadmiumchloride are discussed for each test organism.

3.3.7.1 Sediment toxicity test with *L. variegatus*

The following concentrations were tested in the 28-day sediment toxicity test with *L. variegatus*: 0.008, 0.039, 0.2, 0.98, 5, 25, 123 mg kg^{-1} cadmium. Only one replicate per concentration was tested.

3.3.7.1.1 Number of worms The number of worms after exposure to various concentrations of cadmiumchloride is shown in figure 3.35. There was an obvious difference in the number of worms of controls and solvent controls. Only one replicate per concentration was tested. The number of worms found in controls was 42% lower than in solvent controls. The difference occurs within the test systems variation, for which a maximum coefficient of variance of 41% was found (see details in 3.9.1.1 on page 125). A maximum number of 84 worms was observed in historical controls of performed tests. Therefore, the high variation between control and solvent control is common. No worms were found in the 123 mg kg^{-1} cadmium treatment after 28 days . Worm numbers were reduced by 87% in the 25 mg kg^{-1} treatment compared to the solvent control. There was a clear concentration effect relationship from concentration 5 to 123 mg kg^{-1} cadmium. There was no real concentration effect relationship for the lower concentrations from 0.008 to 0.98 mg kg^{-1}. For the endpoint total worm number, NOEC/LOEC values of 5/25 mg kg^{-1} were estimated according to the method described

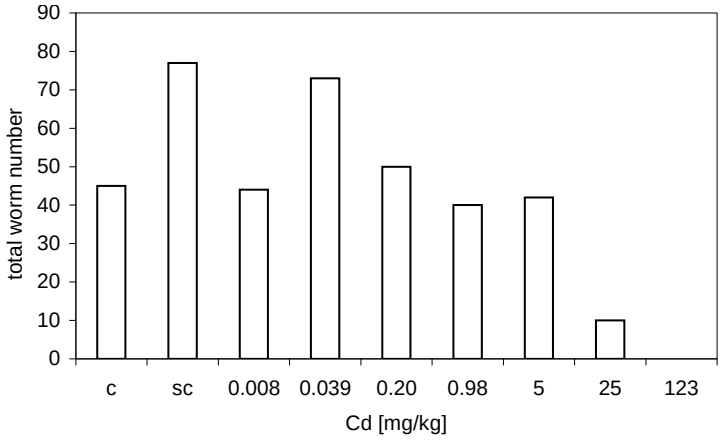


Figure 3.35: Total number of worms after 28 d exposition with cadmiumchloride, only one replicate per concentration

in section 2.4.4 on page 17. An EC_{10} of 0.17 $mg\,kg^{-1}$ and an EC_{50} of 4.39 $mg\,kg^{-1}$ were calculated using probit analysis.

3.3.7.1.2 Biomass Total biomass of *L. variegatus* after exposure to various concentrations of cadmiumchloride is shown in figure 3.36. There was an obvious difference in worm biomass of controls and solvent controls. The worm biomass of controls was 52% lower than of solvent controls. The difference occurs within the test systems variation, for which a maximum coefficient of variance in controls of 41% was found (see details in 3.9.1.2 on page 126). A maximum dry weight of 70 mg was observed in historical controls of performed tests. Therefore, the high variation of biomass between control and solvent control is common. No worms were found in the 123 $mg\,kg^{-1}$ cadmium treatment after 28 days. Worm biomass was reduced by 81% in the 25 $mg\,kg^{-1}$ treatment compared to the solvent control. The biomass also reflects the concentration effect relationship that was observed for the total worm number from concentration 5 to 123 $mg\,kg^{-1}$. For the endpoint biomass, NOEC/LOEC values of 5/25 $mg\,kg^{-1}$ were estimated according to the method described in section 2.4.4 on page 17. An EC_{10} of 4.1 $mg\,kg^{-1}$ and an EC_{50} of 15.0 $mg\,kg^{-1}$ were calculated using probit analysis.

3.3.7.1.3 Summary of *L. variegatus* sediment toxicity test with cadmiumchloride EC_Xvalues with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.16 on page 85 (in $mg\,kg^{-1}$) and in table 3.17 on page 86 (in $\mu mol\,kg^{-1}$). For both endpoints, NOEC/LOEC values of 5/25 $mg\,kg^{-1}$ were derived. The EC_{50} for the endpoint number of worms of 4.4 $mg\,kg^{-1}$ was lower by a factor of 3.4 than for the endpoint biomass. This is due to the relatively lower worm number compared to controls at the lower concentrations. Biomass was relatively higher than worm number at low concentrations.

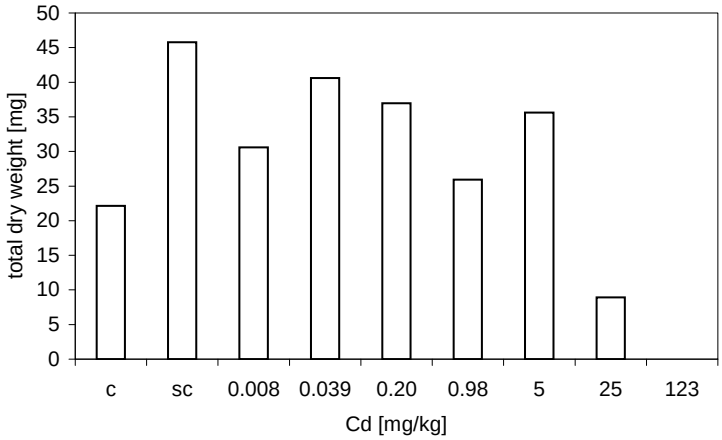


Figure 3.36: Total dry weight of *L. variegatus* after 28 d exposition with cadmiumchloride, only one replicate per concentration

3.3.7.2 Sediment toxicity test with *C. riparius*

The following concentrations were tested in the 28-day sediment toxicity test with *C. riparius*: 0.012, 0.12, 1.2, 12, 122 mg kg^{-1} cadmium.

3.3.7.2.1 Emergence Total emergence of *C. riparius* after exposure to various concentrations of cadmiumchloride is shown in figure 3.37. There was no significant difference in

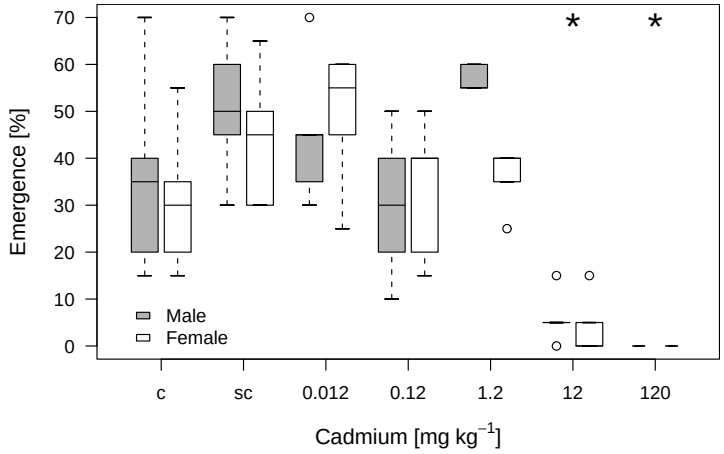


Figure 3.37: Total emergence of *C. riparius* after exposure to various concentrations of cadmiumchloride, c = control, sc = solvent control, * = significantly different to pooled controls (p = 0.05, Williams test)

total emergence of controls and solvent controls. There was a clear concentration effect relationship from 1.2 to 120 mg kg^{-1}. No midges emerged in the highest concentration (120 mg kg^{-1} Cd). Emergence of male and female midges was significantly reduced according to the Williams test (p = 0.05) in the 12 and 120 mg kg^{-1} treatment. A NOEC/LOEC of 1.2/12

$mg\,kg^{-1}$ was derived for the endpoint emergence of male and female midges. An EC_{10} of 4.4 $mg\,kg^{-1}$ and an EC_{50} of 8.2 $mg\,kg^{-1}$ were calculated using probit analysis for the endpoint emergence of male midges. For the emergence of female midges, values were 4.0 and 9.1 $mg\,kg^{-1}$, respectively.

Cumulative emergence curves of midges are shown in figure 3.38. An obvious time shift

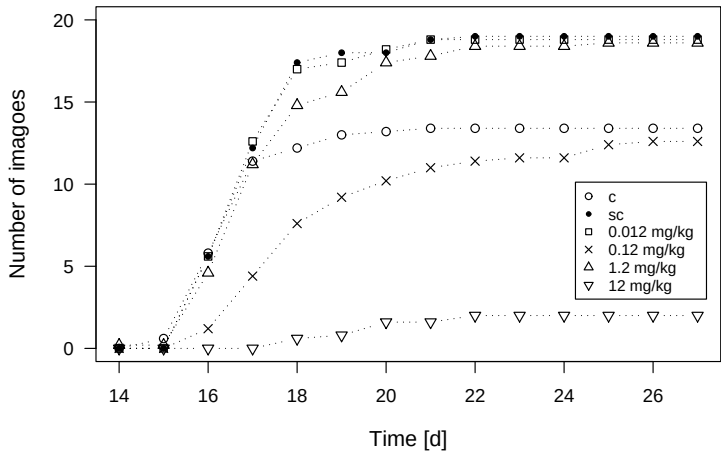


Figure 3.38: Cumulative emergence of *C. riparius* after exposure to various concentrations of cadmiumchloride, c = control, sc = solvent control, mean of all replicates

of the cumulative emergence curves can be observed for treatments 0.12 and 12 $mg\,kg^{-1}$. Statistically significant differences can be observed by comparing the calculated development rates, which are described in detail in the following section 3.3.7.2.2.

3.3.7.2.2 Development rate The development rate of midges is shown in figure 3.39. There was no significant difference in the development rate of controls and solvent controls. No midges emerged in the highest concentration tested. A concentration effect relationship was observed for the endpoint development rate for the tested concentrations. Development rates of treatments 0.12, 1.2, and 12 $mg\,kg^{-1}$ were significantly lower than pooled controls (p = 0.05, Williams Test). For the development rate of male and female *C. riparius*, NOEC/LOEC values of 0.012/0.12 $mg\,kg^{-1}$ were derived.

3.3.7.2.3 Biomass Individual body dry weight of emerged male and female midges is shown in figure 3.40. There was no significant difference in individual and total biomass of controls and solvent controls. Individual body dry weight of both male and female *C. riparius* was not significantly different in any treatments with emergence compared to pooled controls. NOEC/LOEC values for the individual dry weight are 12/120 $mg\,kg^{-1}$.

For total biomass of female *C. riparius*, NOEC/LOEC values of 1.2/12 $mg\,kg^{-1}$ were derived. An EC_{10} of 4.2 $mg\,kg^{-1}$ and an EC_{50} of 7.7 $mg\,kg^{-1}$ were calculated using probit

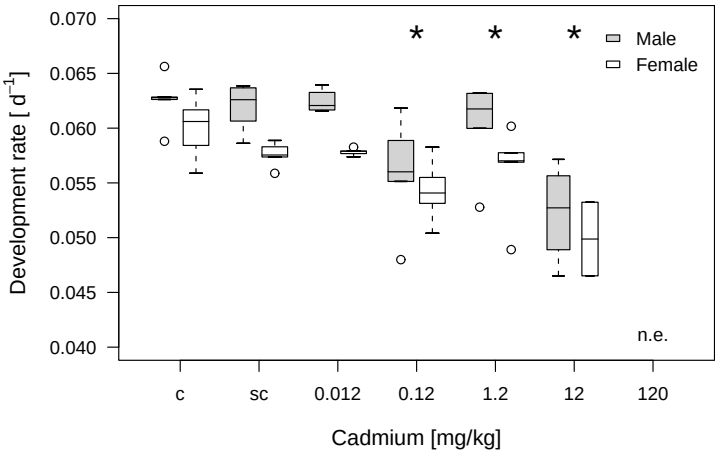


Figure 3.39: Development rate of *C. riparius* after exposure to various concentrations of cadmiumchloride, c = control, sc = solvent control, n.e. = no emergence, * = significantly different to pooled controls (p = 0.05, Williams test)

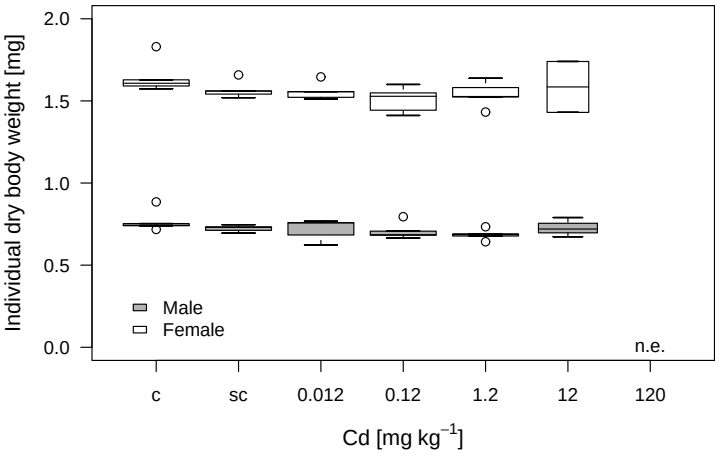


Figure 3.40: Individual body dry weight of male and female imagoes after 28 d exposition with cadmiumchloride

analysis for the endpoint total dry weight of male midges. For female midges, values were 4.0 and 7.2, respectively.

3.3.7.2.4 Summary of *C. riparius* sediment toxicity test with cadmiumchloride

EC_Xvalues with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.18 on page 88 (in $mg\,kg^{-1}$) and in table 3.19 on page 91 (in $\mu mol\,kg^{-1}$). The lowest NOEC/LOEC values of 0.012/0.12 $mg\,kg^{-1}$ were derived for the endpoints development rate of male and female *C. riparius*. Total emergence and total dry weight were not inhibited up to 1.2 $mg\,kg^{-1}$. EC_{50} values of total emergence and total dry weight ranging from 7.2 to 9.1 $mg\,kg^{-1}$ coincided with each other. EC_Xcalculation was not possible for development rates. The endpoint development rate turned out to be the most sensitive endpoint

in the sediment toxicity test with cadmiumchloride. Reduced development rates, and thus a time shift in emergence at these concentration levels, indicated the likelihood for a negative populational impact.

3.3.7.3 Summary of sediment toxicity tests with cadmiumchloride

The following concentrations are referred to cadmium. The lowest effect concentration of the tested invertebrates was observed for *C. riparius* for the endpoint development rate. The derived NOEC/LOEC for this endpoint was 0.07/0.7 μmol kg^{-1} (0.012/0.12 mg kg^{-1}). This is lower by a factor of 37 than the lowest EC_{50} value of 24 μmol kg^{-1} (4.4 mg kg^{-1}) for *L. variegatus*. For *L. variegatus*, the EC_{50} for total worm number was lower by a factor of 3.4 than for biomass. Differences in invertebrate sensitivity are higher than factor of 10, which is considered a large difference (see detailed description for definitions of differences in species sensitivity in section 3.3.10 on page 93). For *C. riparius*, EC_{50} values of the other endpoints ranged from 39.2 to 49.8 μmol kg^{-1}(7.2 to 9.1 mg kg^{-1}). The observed effect on development rate, which results in a shift of emergence time, might not have such a populational impact as a reduction in emergence rate or biomass. *C. riparius* would be the least sensitive species of the tested invertebrates to cadmiumchloride when using the similar endpoints biomass and number of individuals and/or emergence rate for interspecies comparison.

3.3.8 Benzo-[a]-pyrene

Following, the results of the sediment toxicity tests with B(a)P are discussed for each test organism.

3.3.8.1 Sediment toxicity test with *L. variegatus*

The following concentrations were tested in the 28-day sediment toxicity test with *L. variegatus*: 0.064, 0.032, 1.6, 8, 40, 200, 1000 mg kg^{-1} B(a)P. Only one replicate per concentration was tested.

3.3.8.1.1 Number of worms The number of worms after exposure to various concentrations of benzo-[a]-pyrene is shown in figure 3.41. There was no obvious difference in the number of worms of controls and solvent controls. Worm numbers were reduced by 30%, 28%, and 48% in the 40, 200 and 1000 mg kg^{-1} treatments compared to the solvent control. There was a concentration effect relationship from concentration 8 to 1000 mg kg^{-1}. There was no real concentration effect relationship for the lower concentrations from 0.06 to 1.6 mg kg^{-1}. For the endpoint total worm number, NOEC/LOEC values of 200/1000 mg kg^{-1} were estimated according to the method described in section 2.4.4 on page 17 (48% reduction

in the highest concentration was higher than CV of 32%). An EC_{10} of 9 mg kg^{-1} and an EC_{50} of 1116 mg kg^{-1} were calculated using probit analysis.

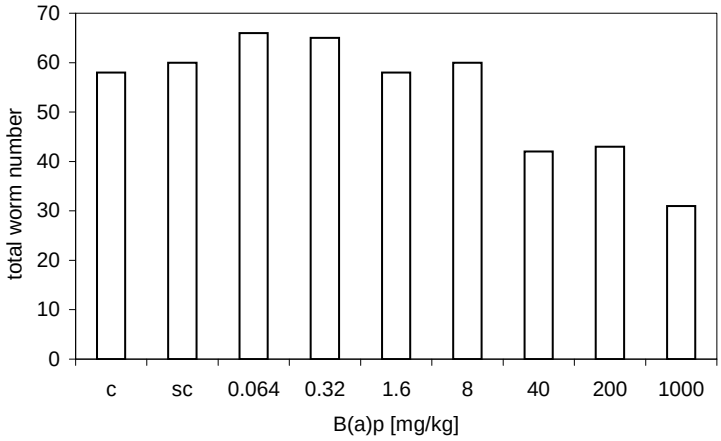


Figure 3.41: Total number of worms after 28 d exposition with B(a)p, only one replicate per concentration

3.3.8.1.2 Biomass Biomass of *L. variegatus* after exposure to various concentrations of benzo-[a]-pyrene is shown in figure 3.42. There was no obvious difference in the worm biomass of controls and solvent controls. Biomass was 46%, 33%, and 75% lower in 40, 200 and 1000 mg kg^{-1} treatments than in the solvent control. The biomass also reflects the concentration effect relationship that was observed for the total worm number from concentration 8 to 1000 mg kg^{-1}. For the endpoint biomass, NOEC/LOEC values of 8/40 mg kg^{-1} were estimated according to the method described in section 2.4.4 on page 17 (reductions in the three highest concentrations were higher than CV of 32%). An EC_{10} of 2 mg kg^{-1} and an EC_{50} of 234 mg kg^{-1} were calculated using probit analysis.

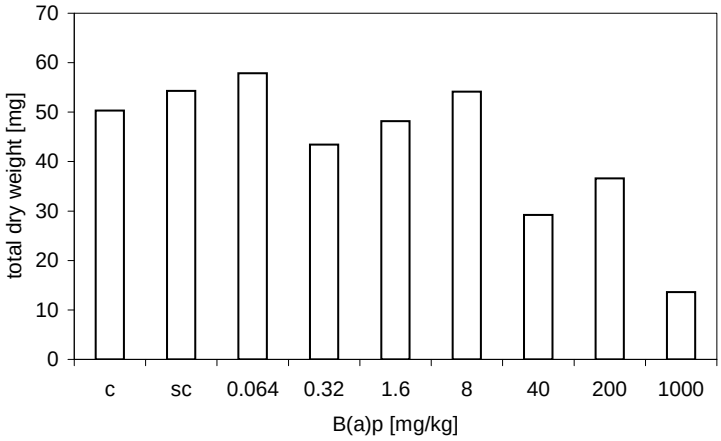


Figure 3.42: Total dry weight of *L. variegatus* after 28 d exposition with B(a)p, only one replicate per concentration

3.3.8.1.3 Summary of *L. variegatus* sediment toxicity test with B(a)P EC_X values with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.16 on page 85 (in $mg\,kg^{-1}$) and in table 3.17 on page 86 (in $\mu mol\,kg^{-1}$). The lowest NOEC/LOEC values of 8/40 $mg\,kg^{-1}$ were estimated for the endpoint biomass. The EC_{50} for the endpoint biomass of 234 $mg\,kg^{-1}$ was lower by a factor of 4.8 than for the endpoint number of worms. This is due to the relatively lower biomass compared to controls at the lower concentrations. Worm number was relatively higher than worm biomass at low concentrations.

3.3.8.2 Sediment toxicity test with *C. riparius*

The following concentrations were tested in the 28-day sediment toxicity test with *C. riparius*: 0.1, 1, 10, 100, 1000 $mg\,kg^{-1}$ B(a)P. Only one replicate per concentration was tested.

3.3.8.2.1 Emergence Total emergence of *C. riparius* after exposure to various concentrations of benzo-[a]-pyrene is shown in figure 3.43. There was no significant difference in total emergence of controls and solvent controls. Results show no clear concentration effect relationship for the endpoint total emergence, even though the highest concentration exhibited the lowest emergence. The highest concentration (1000 $mg\,kg^{-1}$) tested was the observed NOEC. An EC_X calculation using probit analysis was not possible due to mathematical reasons.

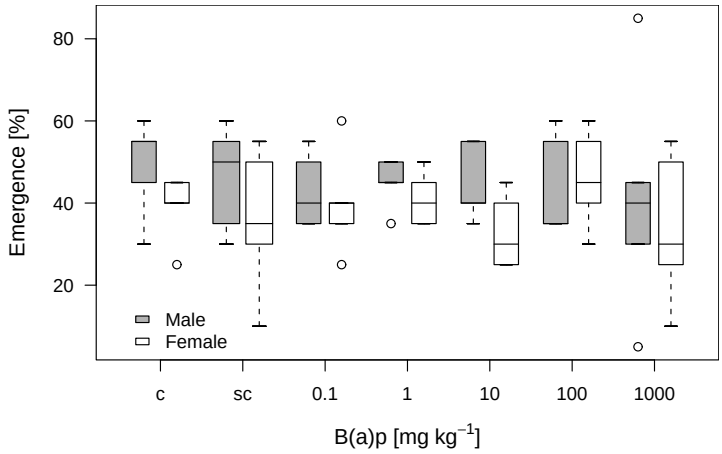


Figure 3.43: Total emergence of *C. riparius* after exposure to various concentrations of B(a)p, c = control, sc = solvent control

Cumulative emergence curves of midges are shown in figure 3.44. No obvious time shift of the cumulative emergence curves was observed. The smallest number of imagoes emerged in the highest treatment. Statistically significant time shift can be observed by comparing the calculated development rates, which are described in detail in the following section 3.3.8.2.2.

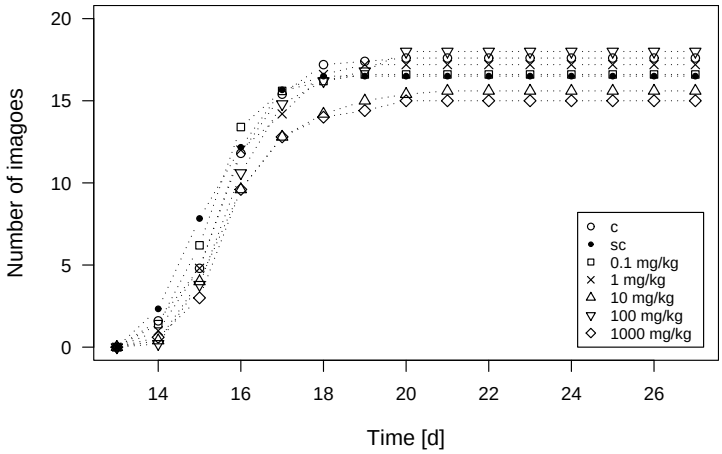


Figure 3.44: Cumulative emergence of *C. riparius* after exposure to various concentrations of B(a)p, c = control, sc = solvent control, mean of all replicates

3.3.8.2.2 Development rate The development rate of midges is shown in figure 3.45. There was no significant difference in the development rate of controls and solvent controls.

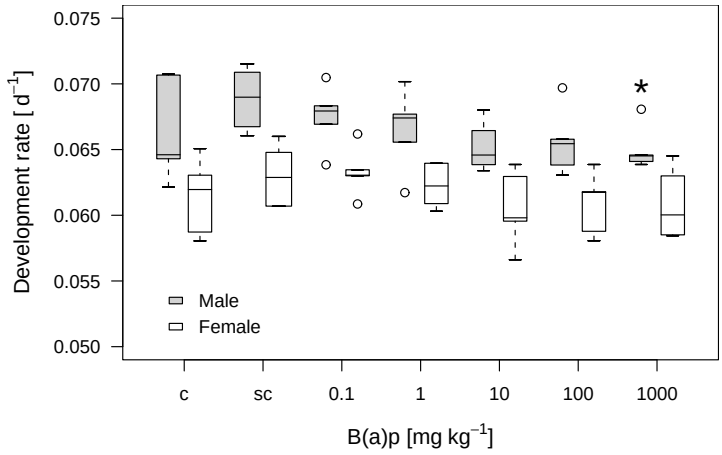


Figure 3.45: Development rate of *C. riparius* after exposure to various concentrations of B(a)p, c = control, sc = solvent control, * = significantly different to controls (p = 0.05, Williams test)

No midges emerged in the highest concentration tested. A clear but not significant concentration effect relationship was observed for the endpoint development rate for the tested concentrations. Development rates are lower with increasing concentrations. But only the development rates of male *C. riparius* in the 1000 mg kg^{-1} treatment were significantly lower than pooled controls (p = 0.05, Williams Test). For the development rate of male *C. riparius*, NOEC/LOEC values of 100/1000 mg kg^{-1} were derived. The highest concentration was the observed NOEC for female *C. riparius*.

3.3.8.2.3 Biomass Individual body dry weight of emerged male and female midges is shown in figure 3.46. There was no significant difference in individual body dry weight of

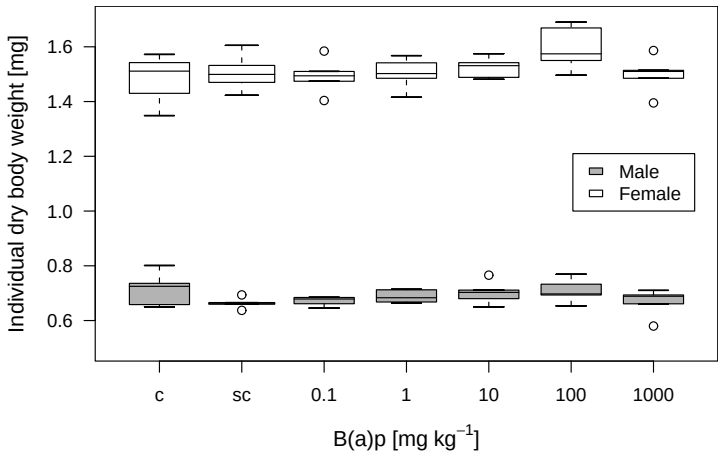


Figure 3.46: Individual body dry weight of male and female imagoes after 28 d exposition with B(a)p, * = significantly different to solvent control (p = 0.05, Williams test)

midges of controls and solvent controls. Individual body dry weight of both male and female *C. riparius* was not significantly different in any treatments compared to pooled controls. Results show no clear concentration effect relationship for the endpoint individual body dry weight. The highest concentration (1000 mg kg^{-1}) tested was the observed NOEC.

Total biomass of both male and female *C. riparius* showed no significant differences to pooled controls. Results show no clear concentration effect relationship for the endpoint total biomass. The highest concentration (1000 mg kg^{-1}) tested was the observed NOEC. An EC_X calculation using probit analysis was not possible due to mathematical reasons.

3.3.8.2.4 Summary of *C. riparius* sediment toxicity test with B(a)P EC_X values with lower and upper 95% confidence limits and NOEC/LOEC values are summarized in table 3.18 on page 88 (in mg kg^{-1}) and in table 3.19 on page 91 (in μmol kg^{-1}). The lowest NOEC/LOEC values of 100/100 mg kg^{-1} were derived for the endpoint development rate of male *C. riparius*. Total emergence, dry weight of imagoes, and development rate of female *C. riparius* were not inhibited up to the highest concentration (1000 mg kg^{-1}) tested. EC_X calculation was not possible for any of the observed endpoints. The endpoint development rate turned out to be the most sensitive endpoint in the sediment toxicity test with B(a)P. Reduced mean development rate, and thus a time shift in emergence at these concentration levels, indicated the likelihood for a negative populational impact. But these NOEC/LOEC levels are very high and are not likely to be found in natural environments.

3.3.8.3 Summary of sediment toxicity tests with B(a)P

L. variegatus was the most sensitive of the two invertebrates in the sediment toxicity test with B(a)P. The lowest EC_{50} value of 930 $\mu mol\,kg^{-1}$ (234 $mg\,kg^{-1}$) for *L. variegatus* was lower by a factor of 4 than for *C. riparius*. For *L. variegatus*, the endpoint biomass was 3.4 times more sensitive than the total number of organisms at the end of the exposure period, whereas for *C. riparius*, only the development rate of male midges was inhibited at the highest tested concentration. Differences in invertebrate sensitivity are lower than factor of 5, which is considered a very small difference (see detailed description for definitions of differences in species sensitivity in section 3.3.10 on page 93).

3.3.9 Effect data of sediment toxicity tests

To limit expenses for chemical analysis, only 3 concentrations of the tested concentrations were analyzed for each test. For all tests with measured concentrations that differed $\pm$ 20% from nominal concentrations, EC_X and NOEC/LOEC were corrected and recalculated. Effective concentrations for EC_X-values are calculated as follows: If not otherwise stated, EC_X-values were corrected by a relative factor. The arithmetic mean of measured concentrations at the start and end of the test was calculated for each measured concentration. Assuming a first order kinetic degradation/ disappearance process over the test period, a geometric mean was calculated out of these values to get the relative factor by which nominal concentrations were corrected. This procedure was done because not all concentrations were analyzed.

Measured values were normally used to calculate effective NOEC/LOEC values. If the concentration of NOEC or LOEC was not analyzed, values were estimated according to the method used for EC_X correction.

3.3.9.1 Overview of sediment toxicity data of *L. variegatus*

Sediment toxicity data of *L. variegatus* are summarized in tables 3.16 (in $mg\,kg^{-1}$) and 3.17 (in $\mu mol\,kg^{-1}$). The lowest effect concentration was observed for TBT-Cl followed by cadmium and PCP, which were of equal toxicity. Then followed 3,4-DCA, DDT, TNT, 2,4-DCP, and B(a)P (also see figure 3.47 on page 94). The tested substances cover a wide range of toxicity. EC_{50} values (if EC_X calculation was not possible, the lowest LOEC was used) of the most and the least toxic substance are by a factor of 310 different (based on $\mu mol\,l^{-1}$). This is lower than the difference observed for *C. riparius*.

Only high effects can be attributed as significant in the test system with *L. variegatus* since high variability with mean coefficient of variances for controls and solvent controls ranging from 29% to 32% was observed (see details in section 3.9.1 on page 125). MARCHINI (2002) and MOORE *et al.* (2004) described that in such systems with high variability, only

differences of 10% to 30% can be attributed as significant. Usually, the NOEC derived for such systems is higher than the calculated EC_{10}. For half of the conducted sediment toxicity tests with *L. variegatus*, factors between NOEC and EC_{10} are smaller than 4. Discrepancies of this observation were observed in four of the performed tests. In the sediment toxicity test with 3,4-DCA for the endpoint total dry weight, the EC_{10} was smaller by a factor of 25 than NOEC. Reasons for this large difference were (1) a high variation of solvent controls and treatments (In these cases only large differences can be attributed statistically significant), and (2) the lowest two treatments already showed a reduction in biomass higher than 10% but not significant. The result was that a far smaller EC_{10} value was calculated by probit analysis.

In the sediment toxicity test with B(a)P for the endpoints total number of worms/ biomass the EC_{10} were smaller by a factor of 22/ 4 than NOEC. In this test, only one replicate per concentration was used. NOEC was estimated according to the method described in section 2.4.4 on page 17 and should be used only as a rough estimation. NOEC/LOEC values of 200/1000 $mg\,kg^{-1}$ were estimated because in the 1000 $mg\,kg^{-1}$ treatment the total number of worms was obviously reduced by 48% compared to solvent control. Worm number was already reduced in the 40 $mg\,kg^{-1}$ treatment by 28%/ 30% compared to control/ solvent control but was not as highly reduced in the next higher treatment of 200 $mg\,kg^{-1}$. The threshold of the calculated mean coefficient of variance for controls/ solvent controls of 32%/ 29% (see section 3.9.1 on page 125) was not exceeded for these two treatments. Therefore, a NOEC of 200 $mg\,kg^{-1}$ was roughly estimated. A different picture was seen for the endpoint total dry weight of worms. Biomass was decreased by more than 32% in the three highest concentrations. The decrease was above the mean coefficient of variance for controls. Therefore, a NOEC of 8 $mg\,kg^{-1}$ was roughly estimated.

In the sediment toxicity test with cadmiumchloride for the endpoint total number of worms, the EC_{10} was smaller by a factor of 29 than NOEC. In this test, only one replicate per concentration was used. NOEC was estimated according to the method described in section 2.4.4 on page 17 and should be used only as a rough estimation. Unfortunately, the number of control worms was 42% lower than in solvent controls. For this case, controls and solvent controls were pooled. Four of the five lowest treatments (next to 25 $mg\,kg^{-1}$, which was the second highest of seven treatments) exhibited worm numbers which were more than 10 % lower than the pooled control and solvent control. Consequently, a smaller EC_{10} value was calculated by probit analysis. Differences in total dry weight between the pooled control and solvent control and the five lowest treatments were not as high.

In the UBA-ring sediment toxicity test with PCP for the endpoint total dry weight, the EC_{10} was smaller by a factor of 6.25 than NOEC. The EC_{10} of total number of worms coincides with NOEC. The mean biomass was reduced starting at the lowest concentration 0.05 $mg\,kg^{-1}$ compared to solvent control and control. This reduction was not significant ($p = 0.05$, Williams test). The result was that a smaller EC_{10} value was calculated by probit analysis. The decrease in the total number of worms was not as obvious at low concentrations.

The observed endpoints total worm number and total dry weight were of equal sensitivity

for most of the tested substances, which were 2,4-DCP, 3,4-DCA, PCP, TBT, and TNT. Dry weight of worms was the most sensitive endpoint in the sediment toxicity test with B(a)P. The endpoint total number of worms was the most sensitive endpoint for DDT and cadmium.

Table 3.16: Summary of nominal and effective sediment toxicity data for *L. variegatus* ($mg\,kg^{-1}$), lower and upper confidence limits are given in parenthesis

Chemical	Endpoint	NOEC / LOEC	EC_{10}	EC_{50}
2,4-DCP	total dry weight	40 / 200 ×	70.0 (69.9-70.2)	120.3 (120.2-120.5)
	total number worms	40 / 200 ×	30 (6.1-52)	102 (61-165)
2,4-DCP eff.	total dry weight	1.3 / 5.4 ×	2.17 (2.17-2.17)	3.7 (3.7-3.7)
	total number worms	1.3 / 5.4 ×	0.96 (0.19-1.61)	3.2 (1.9-5.1)
3,4-DCA	total dry weight	5 / 25 [a]	0.2 [a]	26 [a]
	total number worms	5 / 25 [a]	1.4 [a]	27 [a]
3,4-DCA eff.	total dry weight	0.04 / 0.1 [a]	0.01 [a]	0.15 [a]
	total number worms	0.04 / 0.1 [a]	0.02 [a]	0.16 [a]
4,4-DDT	total dry weight	71 / 500	159.6 (159.3-159.9)	300.5 (300.3-300.8)
	total number worms	10 / 71	10.1 (4.3-17.3)	102.9 (74.8-142.7)
4,4-DDT eff.	total dry weight	34 * / 478	75.8 (75.7-76.0)	142.8 (142.6-142.9)
	total number worms	2.8 / 34 *	4.8 (2.0-8.2)	48.9 (35.5-67.8)
B(a)p	total dry weight	8 / 40 ×	2	234
	total number worms	200 / 1000 ×	9 (0.05-42)	1116 (372-27943)
B(a)p eff.	total dry weight	5.9 / 30.2 * ×	1.5	177
	total number worms	151 * / 844 ×	7 (0.04-32)	843 (281-21097)
Cd	total dry weight	4.9 / 24.5 ×	4.1 (0.1-9.0)	15 (5.7-47.9)
	total number worms	4.9 / 24.5 ×	0.17	4.4
PCP (test 1)	total dry weight	6.25 / 31.25	1	7.7
	total number worms	6.25 / 31.25	6.2 (0.1-12.3)	13.1 (4.7-66)
PCP (test 1) eff.	total dry weight	3.4 / 16.9	0.54	3.8
	total number worms	3.4 / 16.9	3.3 (0.05-6.7)	7 (2.5-35.8)
PCP (test 2)	total dry weight		8.9 (8.8-8.9)	12.9 (12.6-13.2)
	total number worms		13.7 (13.7-13.7)	17.9 (17.9-17.9)
TBT-Sn	total dry weight	0.3 / 1.5 ×	0.7	1
	total number worms	0.3 / 1.5 ×	0.72 (0.67-0.76)	1.12 (1.09-1.14)
TNT	total dry weight	100 / 500 ×	172	224
	total number worms	100 / 500 ×	40 (8.0-75)	138 (72.7-283)
TNT eff.	total dry weight	1.9 * / 9.2 ×	3.2	4.1
	total number worms	1.9 * / 9.2 ×	0.74 (0.15-1.4)	2.6 (1.3-5.2)

× = value estimated, only one replicate per concentration,

* = calculated effective concentration, because concentration was not analyzed, a = (OETKEN *et al.*, 2001)

Table 3.17: Summary of nominal and effective sediment toxicity data for *L. variegatus* (μmol kg^{-1}), lower and upper confidence limits are given in parenthesis

Chemical	Endpoint	NOEC / LOEC	EC_{10}	EC_{50}
2,4-DCP	total dry weight	245 / 1227 ×	429.7 (428.6-430.9)	738.2 (737.2-739.2)
	total number worms	245 / 1227 ×	181.6 (37.2-321.4)	627 (372-1013)
2,4-DCP eff.	total dry weight	8.0 / 33.1 ×	13.32 (13.28-13.35)	22.88 (22.85-22.92)
	total number worms	8.0 / 33.1 ×	5.6 (1.15-9.96)	19.4 (11.5-31.4)
3,4-DCA	total dry weight	31 / 154 [a]	1.2 [a]	162 [a]
	total number worms	31 / 154 [a]	8.4 [a]	165 [a]
3,4-DCA eff.	total dry weight	0.25 / 0.62 [a]	0.06 [a]	0.93 [a]
	total number worms	0.25 / 0.62 [a]	0.12 [a]	0.99 [a]
4,4-DDT	total dry weight	199 / 1410	450.2 (449.4-451.1)	847.8 (847.1-848.5)
	total number worms	28 / 199	28.5 (12.1-48.8)	290 (211-403)
4,4-DDT eff.	total dry weight	96 * / 1348	213.8 (213.5-214.3)	402.7 (402.4-403.0)
	total number worms	7.9 / 96 *	13.5 (5.8-23.2)	138 (100-191)
B(a)p	total dry weight	31.7/ 158.5 ×	10.6	930
	total number worms	793 / 3963 ×	39 (0.2-165.2)	4426 (1476-110748)
B(a)p eff.	total dry weight	23.3 / 120 * ×	8.0	702
	total number worms	599 */ 3345 ×	29.4 (0.15-125)	3342 (1114-83615)
Cd	total dry weight	44 / 218 ×	36.4 (1.1-80)	133 (50.7-426)
	total number worms	44 / 218 ×	1.5	39.0
PCP (test 1)	total dry weight	23 / 117	3.8	28.9
	total number worms	23 / 117	23 (0.4-46)	49 (18-247)
PCP (test 1)	total dry weight	12.2 / 63.4	2.1	15.7
eff.	total number worms	12.2 / 63.4	12.5 (0.2-24.9)	26.6 (9.8-134)
PCP (test 2)	total dry weight		33.3 (33.1-33.5)	48.3 (47.2-49.6)
	total number worms		52 (52-52)	67 (67-67)
TBT-Sn	total dry weight	2.5 / 12.3 ×	5.6	8.3
	total number worms	2.5 / 12.3 ×	6.1 (5.7-6.4)	9.4 (9.2-9.6)
TNT	total dry weight	440 / 2201 ×	756	984
	total number worms	440 / 2201 ×	175	605
TNT eff.	total dry weight	8,1 * / 40,7 ×	14	18.2
	total number worms	8,1 * / 40,7 ×	3.2	11.2

× = value estimated, only one replicate per concentration,

* = calculated effective concentration, because concentration was not analyzed, a = (OETKEN *et al.*, 2001)

3.3.9.2 Overview of sediment toxicity data of *C. riparius*

Sediment toxicity data of *C. riparius* are summarized in tables 3.18 (in $mg\,kg^{-1}$) and 3.19 (in $\mu mol\,kg^{-1}$). The lowest effect concentration was observed for 3,4-DCA for the endpoint development rate in experiments of OETKEN *et al.* (2001). The second lowest effect concentration was observed for cadmium followed by DDT, TBT, PCP, 2,4-DCP, TNT, and B(a)P (also see figure 3.47 on page 94). The tested substances cover a wide range of toxicity. EC_{50} values (if EC_X calculation was not possible, the lowest LOEC was used) of the most and the least toxic substance are different by a factor of 9908 (based on $\mu mol\,l^{-1}$).

Only high effects can be attributed as significant for the endpoints total emergence and dry weight in the test system with *C. riparius*, since high variability with mean coefficient of variances for controls and solvent controls ranging from 22% to 47% was observed (see details in section 3.9.2 on page 129). MARCHINI (2002) and MOORE *et al.* (2004) described that in such systems with high variability only differences of 10% to 30% can be attributed as significant. Usually, the NOEC derived for such systems is higher than the calculated EC_{10}. For the endpoints individual dry weight and development rate, smaller differences to controls can be attributed as significant because coefficient of variances were small with mean values ranging from 6.2 to 11.9.

For most of the conducted sediment toxicity tests with *C. riparius*, factors between NOEC and EC_{10} are smaller than 4. Discrepancies of this observation were observed for two endpoints of the sediment toxicity test with PCP and for one endpoint in the test with TNT.

In the sediment toxicity test with PCP for the endpoint total emergence of male *C. riparius*, the EC_{10} was smaller by a factor of 5.3 than NOEC. Reasons for this large difference were (1) a high variation of controls, solvent controls, and treatments resulting in large differences that would be necessary to be significant, and (2) the mean emergence of male midges in the four lowest treatments were already reduced by 5% to 20%. This resulted in a small EC_{10} value calculated by probit compared to the NOEC. For the endpoint total dry weight of male *C. riparius*, the EC_{10} was smaller by a factor of 46 than NOEC. Reasons for this large difference were again a high variation of controls, solvent controls, and treatments resulting in large differences that would be necessary to be significant. Also, the mean dry weights of male midges were already reduced by 17% to 31% in the four lowest treatments.

For 2,4-DCP, DDT, and TBT, all observed endpoints were of nearly equal sensitivity. Total emergence was the most sensitive endpoint in the test with TNT. Individual dry weight was the most sensitive endpoint in the test with PCP. Development rate / EMT_{50} was the most sensitive endpoint in the sediment toxicity tests with cadmium, B(a)P, and 3,4-DCA.

Table 3.18: Summary of nominal and effective sediment toxicity data for *C. riparius* ($mg\,kg^{-1}$), lower and upper confidence limits are given in parenthesis

Chemical	Endpoint	NOEC / LOEC	EC_{10}	EC_{50}
2,4-DCP	development rate female	17.9 / 40		
	development rate male	17.9 / 40		
	emergence female + male	17.9 / 40	23.4	26.8
	emergence female	17.9 / 40	23.4	26.8
	emergence male	17.9 / 40	23.4	26.8
	individual dry weight female	17.9 / 40		
	individual dry weight male	17.9 / 40		
	total dry weight female	17.9 / 40	19.5	26.3
	total dry weight male	17.9 / 40	23.4 (23.4-23.4)	26.8 (26.8-26.8)
2,4-DCP eff.	development rate female	5.2 / 10.4		
	development rate male	5.2 / 10.4		
	emergence female + male	5.2 / 10.4	7.2	8.2
	emergence female	5.2 / 10.4	7.2	8.2
	emergence male	5.2 / 10.4	7.2	8.2
	individual dry weight female	5.2 / 10.4		
	individual dry weight male	5.2 / 10.4		
	total dry weight female	5.2 / 10.4	6.0	8.0
	total dry weight male	5.2 / 10.4	7.2 (7.2-7.2)	8.2 (8.2-8.2)
3,4-DCA	emergence	40 / - [a]		
	EMT50 ×	- / 0.064 [a]		
3,4-DCA eff.	emergence	0.23 / - [a]		
	EMT50 ×	- / 0.003 [a]		
4,4-DDT	development rate female	2.7 / 8.1		
	development rate male	2.7 / 8.1		
	emergence f+m	0.3 / 0.9	0.25 (0.06-0.42)	0.83 (0.54-1.28)
	emergence female	0.9 / 2.7	0.48	1.24
	emergence male	0.3 / 0.9	0.23 (0.19-0.26)	0.62 (0.57-0.66)
	individual dry weight female	0.9 / 2.7		
	individual dry weight male	0.3 / 0.9		
	total dry weight female	0.9 / 2.7	0.43 (0.003-0.78)	1.21 (0.54-2.91)
	total dry weight male	0.3 / 0.9	0.14 (0.0004-0.29)	0.48 (0.18-1.27)
4,4-DDT eff.	development rate female	0.7* / 2.9		
	development rate male	0.7* / 2.9		
	emergence f+m	0.04 / 0.24	0.07 (0.02-0.11)	0.22 (0.14-0.34)
	emergence female	0.24 / 0.7*	0.13	0.33
	emergence male	0.04 / 0.24	0.06 (0.05-0.07)	0.16 (0.15-0.18)
	individual dry weight female	0.24 / 0.7*		
	individual dry weight male	0.04 / 0.24		

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Chemical	Endpoint	NOEC / LOEC	EC_{10}	EC_{50}
	total dry weight female	0.24 / 0.7*	0.11 (0.0008-0.21)	0.32 (0.14-0.77)
	total dry weight male	0.04 / 0.24	0.04 (0.00011-0.08)	0.13 (0.05-0.34)
B(a)p	development rate female	1000 / -		
	development rate male	100 / 1000		
	emergence f+m	1000 / -		
	emergence female	1000 / -		
	emergence male	1000 / -		
	individual dry weight female	1000 / -		
	individual dry weight male	1000 / -		
	total dry weight female	1000 / -		
	total dry weight male	1000 / -		
B(a)p eff.	development rate female	738 / -		
	development rate male	76 / 738		
	emergence f+m	738 / -		
	emergence female	738 / -		
	emergence male	738 / -		
	individual dry weight female	738 / -		
	individual dry weight male	738 / -		
	total dry weight female	738 / -		
	total dry weight male	738 / -		
Cd	development rate female	0.012 / 0.12		
	development rate male	0.012 / 0.12		
	emergence f+m	1.2 / 12	4.16	7.55
	emergence female	1.2 / 12	4.02 (3.99-4.04)	9.13 (9.11-9.14)
	emergence male	1.2 / 12	4.41	8.24
	individual dry weight female	12 / 122		
	individual dry weight male	12 / 122		
	total dry weight female	1.2 / 12	4.03	7.20
	total dry weight male	1.2 / 12	4.22	7.71
PCP	development rate female	50 / 500		
	development rate male	50 / 500		
	emergence f+m	50 / 500	60.7	102.1
	emergence female	50 / 500	61.7(41.0-83.9)	151.9 (113.9-206.8)
	emergence male	50 / 500	9.41	97.4
	individual dry weight female	0.5 / 5		
	individual dry weight male	0.5 / 5		
	total dry weight female	50 / 500	51.5	65.8
	total dry weight male	50 / 500	1.09	45.8
PCP eff.	development rate female	38* / 350		
	development rate male	38* / 350		

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Chemical	Endpoint	NOEC / LOEC	EC_{10}	EC_{50}
	emergence f+m	38* / 350	45.6	76.7
	emergence female	38* / 350	46.4 (30.8-63.0)	114.1 (85.5-155.3)
	emergence male	38* / 350	7.1	73.1
	individual dry weight female	0.4 / 3.8		
	individual dry weight male	0.4 / 3.8		
	total dry weight female	38* / 350	38.7	49.4
	total dry weight male	38* / 350	0.8	34.4
TBT-Sn	development rate female	1.46 / 2.92		
	development rate male	2.92 / 5.84		
	emergence f+m	1.46 / 2.92	0.95 (0.02-1.57)	2.32 (1.20-4.54)
	emergence female	1.46 / 2.92	0.81	2.18
	emergence male	1.46 / 2.92	1.12 (0.32-1.60)	2.45 (1.77-3.39)
	individual dry weight female	1.46 / 2.92		
	individual dry weight male	1.46 / 2.92		
	total dry weight female	1.46 / 2.92	0.83 (0.22-1.22)	1.91 (1.36-2.69)
	total dry weight male	1.46 / 2.92	0.90 (0.49-1.19)	2.03 (1.66-2.48)
TNT	development rate female	80 / 200		
	development rate male	80 / 200		
	emergence f+m	80 / 200	98.3	269.9
	emergence female	200 / -		
	emergence male	200 / -		
	individual dry weight female	200 / -		
	individual dry weight male	200 / -		
	total dry weight female	80 / 200	6.75	1170
	total dry weight male	200 / -		
TNT eff.	development rate female	≈ 0.4 / ≈ 1		
	development rate male	≈ 0.4 / ≈ 1		
	emergence f+m	≈ 0.4 / ≈ 1	≈ 0.5	≈ 1.3
	emergence female	≈ 1 / -		
	emergence male	≈ 1 / -		
	individual dry weight female	≈ 1 / -		
	individual dry weight male	≈ 1 / -		
	total dry weight female	≈ 0.4 / ≈ 1	≈ 0.03	≈ 5.8
	total dry weight male	≈ 1 / -		

a = (OETKEN *et al.*, 2001), $^{\times}$ = value given only for EMT_{50}, which is reciprocal of development rate,
* = calculated effective concentration, because concentration was not analyzed,
$\approx$ = effective concentrations by approximately factor 200 lower than nominal concentrations,
most measurements below limit of quantification except for one concentration each for t0 and t28,
$^{\times}$ = EMT50 is the reciprocal value of development rate

Table 3.19: Summary of nominal and effective sediment toxicity data for *C. riparius* (μmol kg^{-1}), lower and upper confidence limits are given in parenthesis

Chemical	Endpoint	NOEC / LOEC	EC_{10}	EC_{50}
2,4-DCP	development rate female	109.8 / 245.4		
	development rate male	109.8 / 245.4		
	emergence f+m	109.8 / 245.4	143.6	164.4
	emergence female	109.8 / 245.4	143.6	164.4
	emergence male	109.8 / 245.4	143.6	164.4
	individual dry weight female	109.8 / 245.4		
	individual dry weight male	109.8 / 245.4		
	total dry weight female	109.8 / 245.4	119.6	161.3
	total dry weight male	109.8 / 245.4	143.6 (143.6-143.6)	164.4 (164.4-164.4)
2,4-DCP eff.	development rate female	31.9 / 63.8		
	development rate male	31.9 / 63.8		
	emergence female + male	31.9 / 63.8	43.9	50.3
	emergence female	31.9 / 63.8	43.9	50.3
	emergence male	31.9 / 63.8	43.9	50.3
	individual dry weight female	31.9 / 63.8		
	individual dry weight male	31.9 / 63.8		
	total dry weight female	31.9 / 63.8	36.6	49.3
	total dry weight male	31.9 / 63.8	43.9 (43.9-43.9)	50.3 (50.3-50.3)
3,4-DCA	emergence	246.9 / - [a]		
	EMT50 [×]	- / 0.4 [a]		
3,4-DCA eff.	emergence	1.4 / - [a]		
	EMT50 [×]	- / 0.02 [a]		
4,4-DDT	development rate female	7.6 / 22.8		
	development rate male	7.6 / 22.8		
	emergence f+m	0.8 / 2.5	0.71 (0.17-1.18)	2.34 (1.52-3.61)
	emergence female	2.5 / 7.6	1.35	3.5
	emergence male	0.8 / 2.5	0.65 (0.54-0.73)	1.75 (1.61-1.86)
	individual dry weight female	0.8 / 2.5		
	individual dry weight male	0.8 / 2.5		
	total dry weight female	2.5 / 7.6	1.22 (0.008-2.20)	3.41 (1.52-8.21)
	total dry weight male	0.8 / 2.5	0.40 (0.001-0.81)	1.34 (0.50-3.58)
4,4-DDT eff.	development rate female	2.0* / 8.2		
	development rate male	2.0* / 8.2		
	emergence f+m	0.1 / 0.7	0.19 (0.04-0.31)	0.62 (0.40-0.95)
	emergence female	0.7 / 2.0*	0.36	0.93
	emergence male	0.1 / 0.7	0.17 (0.14-0.19)	0.46 (0.43-0.49)
	individual dry weight female	0.7 / 2.0*		

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Chemical	Endpoint	NOEC / LOEC	EC_{10}	EC_{50}
	individual dry weight male	0.1 / 0.7		
	total dry weight female	0.7 / 2.0*	0.32 (0.002-0.58)	0.91 (0.40-2.2)
	total dry weight male	0.1 / 0.7	0.10 (0.0003-0.22)	0.36 (0.13-0.95)
B(a)p	development rate female	3963 / -		
	development rate male	396 / 3963		
	emergence f+m	3963 / -		
	emergence female	3963 / -		
	emergence male	3963 / -		
	individual dry weight female	3963 / -		
	individual dry weight male	3963 / -		
	total dry weight female	3963 / -		
	total dry weight male	3963 / -		
B(a)p eff.	development rate female	2925 / -		
	development rate male	301 / 2925		
	emergence f+m	2925 / -		
	emergence female	2925 / -		
	emergence male	2925 / -		
	individual dry weight female	2925 / -		
	individual dry weight male	2925 / -		
	total dry weight female	2925 / -		
	total dry weight male	2925 / -		
Cd	development rate female	0.11 / 1.1		
	development rate male	0.11 / 1.1		
	emergence f+m	10.9 / 109	37	67
	emergence female	10.9 / 109	35.7 (35.5-35.9)	81.2 (81.0-81.3)
	emergence male	10.9 / 109	39.3	73.4
	individual dry weight female	109 / 1090		
	individual dry weight male	109 / 1090		
	total dry weight female	10.9 / 109	36	64
	total dry weight male	10.9 / 109	37.5	68.6
PCP	development rate female	188 / 1880		
	development rate male	188 / 1880		
	emergence f+m	188 / 1880	227.8	383.5
	emergence female	188 / 1880	232 (154-315)	570 (428-776)
	emergence male	188 / 1880	35	366
	individual dry weight female	1.88 / 18.8		
	individual dry weight male	1.88 / 18.8		
	total dry weight female	188 / 1880	193	247
	total dry weight male	188 / 1880	4.1	172
PCP eff.	development rate female	141* / 1314		
	development rate male	141* / 1314		

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Chemical	Endpoint	NOEC / LOEC	EC_{10}	EC_{50}
	emergence f+m	141* / 1314	171	288
	emergence female	141* / 1314	174 (116-237)	428 (321-583)
	emergence male	141* / 1314	27	275
	individual dry weight female	1.5 / 14.3		
	individual dry weight male	1.5 / 14.3		
	total dry weight female	141* / 1314	145	185
	total dry weight male	141* / 1314	3.1	129
TBT-Sn	development rate female	4.5 / 9.0		
	development rate male	9.0 / 17.9		
	emergence f+m	4.5 / 9.0	2.9 (0.07-4.8)	7.2 (3.7-14.0)
	emergence female	4.5 / 9.0	2.5	6.7
	emergence male	4.5 / 9.0	3.4 (0.99-4.93)	7.5 (5.4-10.4)
	individual dry weight female	4.5 / 9.0		
	individual dry weight male	4.5 / 9.0		
	total dry weight female	4.5 / 9.0	2.5 (0.7-3.7)	5.9 (4.2-8.3)
	total dry weight male	4.5 / 9.0	2.8 (1.5-3.7)	6.2 (5.1-7.6)
TNT	development rate female	352 / 880		
	development rate male	352 / 880		
	emergence f+m	352 / 880	432	1188
	emergence female	880 / -		
	emergence male	880 / -		
	individual dry weight female	880 / -		
	individual dry weight male	880 / -		
	total dry weight female	352 / 880	30	5151
	total dry weight male	880 / -		
TNT eff.	development rate female	$\approx$ 1.6 / $\approx$ 4.1		
	development rate male	$\approx$ 1.6 / $\approx$ 4.1		
	emergence f+m	$\approx$ 1.6 / $\approx$ 4.1	2	5.5
	emergence female	$\approx$ 4.1 / -		
	emergence male	$\approx$ 4.1 / -		
	individual dry weight female	$\approx$ 4.1 / -		
	individual dry weight male	$\approx$ 4.1 / -		
	total dry weight female	$\approx$ 1.6 / $\approx$ 4.1	0.14	24
	total dry weight male	$\approx$ 4.1 / -		

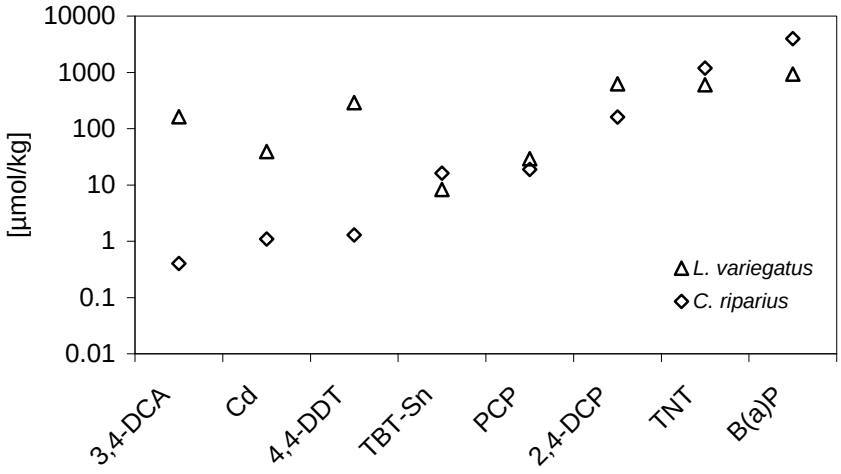
a = (OETKEN *et al.*, 2001), $^{\times}$ = value given only for EMT_{50}, which is reciprocal of development rate
* = calculated effective concentration, because concentration was not analyzed,
$\approx$ = effective concentrations by approximately factor 200 lower than nominal concentrations, most measurements below limit of quantification except for one concentration each for t0 and t28,
$^{\times}$ = EMT50 is the reciprocal value of development rate

3.3.10 Comparative discussion of sediment toxicity data

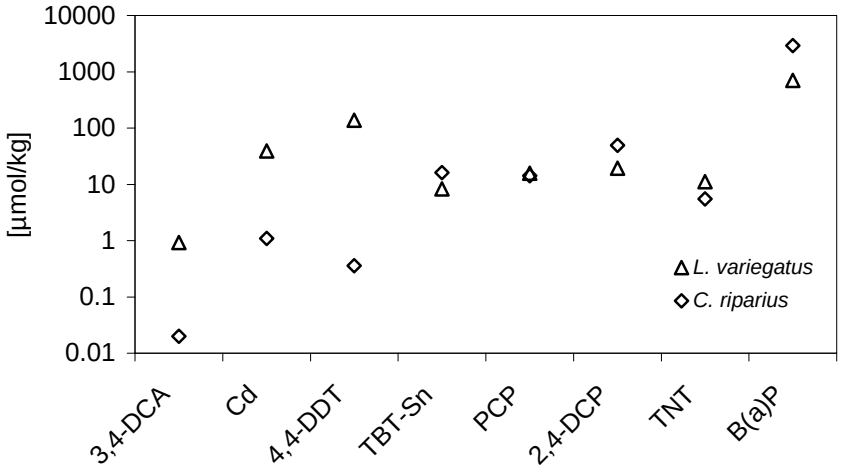
As outlined in section 3.1.2.2 on page 27, evidence exists that first instar larvae are more sensitive than older larval stages in acute toxicity tests. For 10-day sediment bioassays, NAYLOR & HOWCROFT (1997) found no evidence for loss of sensitivity when the test is started with

second as opposed to first instar larvae. However, to assess the effects on chironomids over the course of the complete life cycle, larvae of post-hatching stage (< 24 hour old) were exposed.

The lowest EC_{50}/ LOEC data of sediment toxicity tests of the two invertebrates are shown in figures 3.47(a) (based on nominal concentrations) and 3.47(b) (based on effective concentrations). For some endpoints, it was not possible to calculate EC_{50}. For these cases the LOEC was used. Substances are ranked by sensitivity of *C. riparius* based on nominal concentrations.



(a) based on nominal concentration



(b) based on effective concentration

Figure 3.47: Lowest EC_{50}/ LOEC data of *C. riparius* and *L. variegatus* sediment toxicity tests, substances are ranked by sensitivity of *C. riparius*

There are large differences in the effect concentrations of the selected substances. Following, substances are ranked by difference in species sensitivity. The ranking was done according to the method used for acute toxicity data (see section 3.1.3). There are no or very small differences when the factor of difference in species sensitivity (f_{dss}) is lower or equal to 5. Factors higher than 5 but smaller than 10 are considered a small difference. Values above

10 are considered a large difference. There are similarities in species sensitivity for five of the eight tested substances. Differences smaller than a factor of 5 were observed for PCP, TBT, TNT, 2,4-DCP, and B(a)P with f_{dss} of 1.5/ 1.1, 1.9/ 1.9, 2.0/ 2.0, 3.9/ 2.5, and 4.3/ 4.2, respectively, based on nominal/ effective concentrations. A difference by a factor higher than 5 but lower than 10 was not observed for any substance for the tested invertebrates. For all the other substances, differences between the lowest and highest value are higher than a factor of 10, which are considered to be a large difference according to the above definition. The largest differences in species sensitivity were observed for DDT, 3,4-DCA, and Cd with f_{dss} of 223/ 383, 405/ 47, and 35/ 35, respectively, for nominal/ effective concentrations. As was observed for acute toxicity data comparison (see section 3.1.3 on page 27), very small differences in sediment toxicity of 2,4-DCP and TNT between *C. riparius* and *L. variegatus* were observed. The toxicity of PCP is nearly the same for the tested invertebrates.

Comparison of effect data for *C. riparius* and *L. variegatus* for the tested chemicals indicates that no species was consistently the most sensitive to the eight chemicals. *C. riparius* is the most sensitive species for all three substances (cadmium, 3,4-DCA, and DDT) with f_{dss} higher than 10. Further, *C. riparius* was twice as sensitive as *L. variegatus* towards TNT. *L. variegatus* was more sensitive than *C. riparius* for B(a)P, 2,4-DCP, and TBT, which are three of the eight tested chemicals. However, differences were smaller than a factor of 5. If an f_{dss} smaller than 5 is disregarded, then *C. riparius* is more sensitive than *L. variegatus*. This result is partly not expected from the findings of SIMKISS *et al.* (2001). It was reported that *C. riparius* are accumulating much less of a body load than *L. variegatus*, which may possibly be explained by their differing ability to detoxify xenobiotics. *C. riparius* is able to detoxify a wide range of xenobiotics (SIMKISS *et al.*, 2001). Thus, a generally lower sensitivity of *C. riparius* would be expected.

The lowest EC_{50}/ LOEC values of *C. riparius* and *L. variegatus* sediment toxicity tests are shown according to chemicals log K_{ow} in figure 3.48. As expected, there is no relation between toxicity and lipophilicity since toxicity is dependent on the dose of the substance.

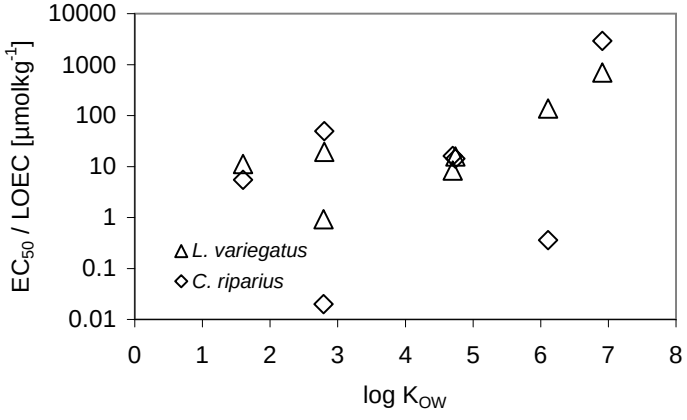
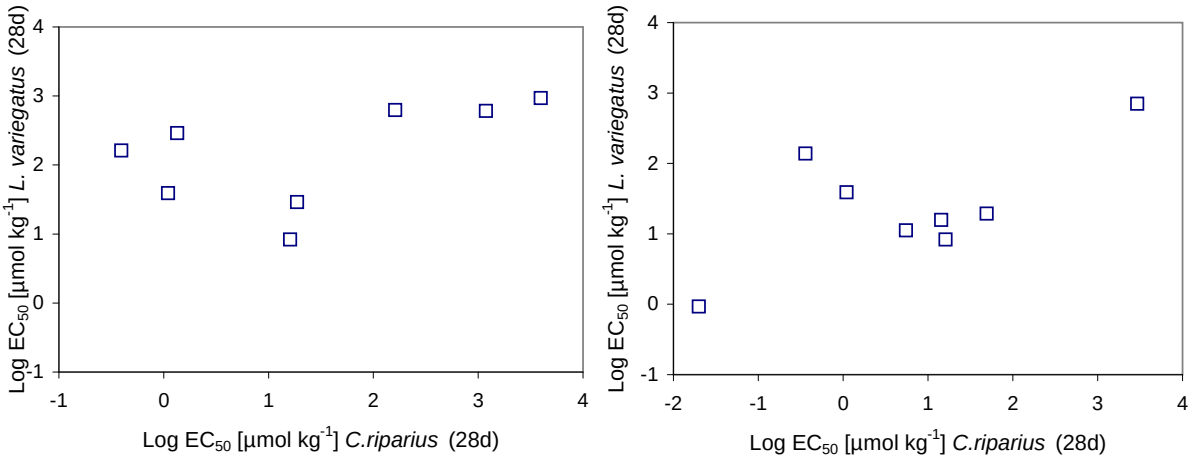


Figure 3.48: Lowest EC_{50}/ LOEC values of *C. riparius* and *L. variegatus* sediment toxicity tests in comparison to log K_{ow}

Interspecies correlation of sediment toxicity data

The third main aspect of this study was to investigate whether predictions from sediment toxicity data of one organism to another would be possible. Therefore, sediment toxicity data of *C. riparius* were correlated with the data of *L. variegatus* (figure 3.49). Because the data were not bivariate normally distributed, the Spearman's rank correlation test was used for correlation analysis. Data were not transformed before the correlation analysis. There is no significant correlation between the lowest EC_{50} data of *C. riparius* and *L. variegatus* ($\rho = 0.33$, $p = 0.21$ (based on nominal concentrations), $\rho = 0.60$, $p = 0.06$ (based on effective concentrations)). Thus sediment toxicity data may not be extrapolated from one to the other sediment species. *C. riparius* was more sensitive than *L. variegatus* in five cases of the eight tested substances. In three of those five cases, *C. riparius* was more than 10 times more sensitive than *L. variegatus*. Factors were 405/ 47, 223/ 383 and 36/ 36 for 3,4-DCA, DDT and cadmium, respectively for nominal/ effective data. For all the other tested chemicals, factors of difference in sensitivity of the two organisms were less than 5. If the sensitivity differences with factors lower than 5 are disregarded, *C. riparius* was more sensitive than *L. variegatus* in the sediment toxicity tests for the tested substances.



(a) based on nominal concentration (b) based on effective concentration

Figure 3.49: Correlation of 28 d EC_{50} of *C. riparius* and *L. variegatus*, if EC_{50} could not be calculated the lowest LOEC was used

Comparative discussion with literature data

Following, sediment toxicity data of this study are compared and discussed with literature data.

In a study performed by DAY *et al.* (1998) (artificial sediment with half natural sediment and feeding during exposure period), survival of *C. riparius* was not reduced below 70% up to 12.3 $mg\,kg^{-1}$ TBT-Sn after a 10-day exposure. In the same study, a NOEC / LOEC of 1.7 / 2.7 $mg\,kg^{-1}$ and an EC_{50} for endpoint growth of 4.3 $mg\,kg^{-1}$ were reported. These values are

about in the range of this study (LOEC for dry weight of female *C. riparius* = 2.9 $mg\,kg^{-1}$). A nominal LC_{50} on the endpoint mortality of 0.112 $mg\,kg^{-1}$ TBT-Sn was found by PEDINA (2001). This test was performed according to OECD test guideline 218 (OECD, 2000) with external feeding during exposition. This value is 16 times lower than the lowest EC_{50} for the endpoint total dry weight of female in this study. This surprising variation might be explained by the very different sediment composition of the two artificial sediments. Artificial sediment composition of this study differs compared to OECD test guideline 218. According to OECD test guideline 218, larvae were offered artificial sediment (5% peat, kaolin clay, sand, and calcium carbonate), and food (TetraPhyll® or TetraMin®) was added during the exposure period. STUIJFZAND *et al.* (2000) observed positive effects of particulate organic matter on the growth of *C. riparius*; thus, high food levels allow this species to thrive under chemical stressors (DE HAAS *et al.*, 2004, 2005). But quantity of food provided *ad libitum* in the study of PEDINA (2001) was not a limiting factor, but that food quality was likely to play a significant role (STUIJFZAND *et al.*, 2000). The difference in sensitivity of the two test systems may partly be caused by a compensation of toxic effects by nutritional effects of particulate organic matter within the sediment of this study. This leads to the hypothesis that the food composition of sediment of this study was of higher quality than that of studies according to OECD test guideline 218. In a sediment toxicity test with quartz sand only containing no organic matter with external feeding, VOGT *et al.* (in press) reported LC_{50} of 8.27 $\mu g\,kg^{-1}$ (only 6% of nominal concentration), which is 231 times lower than the EC_{50} for endpoint total dry weight of this study. Since there was nearly no organic matter within the sediment that usually adsorb TBT, bioavailability was very high compared to this study and may explain the lower LC_{50} value. Further, mean average emergence is about 3 days (male midges) and 6 days (female midges) later than in this study, which indicates a food limited system that may have enhanced the toxicity of TBT. Larvae were fed only 0.5 mg TetraMin® per larvae and day (VOGT, personal communication), which is little smaller than the minimum daily feeding level (0.6 mg per larvae and day) to avoid density effects and food limitation in reproduction tests (PERY *et al.*, 2002). For *H. azteca* an EC_{50} 14-day growth of 1.4 $mg\,kg^{-1}$ TBT-Sn (DAY *et al.*, 1998) and an EC_{50} 10 weeks for reproduction of 0.238 $mg\,kg^{-1}$ TBT-Sn (BARTLETT *et al.*, 2004b) were reported. LC_{50} values of 1.46 $mg\,kg^{-1}$ for survival after 4 weeks exposure and 0.93 $mg\,kg^{-1}$ for survival after 10 weeks exposure were observed (BARTLETT *et al.*, 2004b). Further, an EC_{50} (28 days) for the endpoint number of adults of 0.24 $mg\,kg^{-1}$ was reported by FIEDLER (personal communication). These findings indicate that *H. azteca* is more sensitive towards TBT than *C. riparius* and *L. variegatus* if the LC_{50} value for *C. riparius* reported by VOGT *et al.* (in press) is disregarded.

For *Chironomus tentans* (second instar) a 10-day LC_{50} of 176 $mg\,kg^{-1}$ TNT and for *H. azteca* (10 to 12 days old juvenile) a 10-day LC_{50} of 28.9 $mg\,kg^{-1}$ TNT (both values based on calculated TNT based on its degradation products) were found by STEEVENS *et al.* (2002) with only a 24-hour sediment equilibration period. A 10-day LC_{50} for *C. tentans* of 56.2 $mg\,kg^{-1}$ TNT (based on the measured initial sum of parent and breakdown products) was calculated by LOTUFO & FARRAR (2005) with only a 2- to 3-hours equilibration period. The reported

values for *C. tentans* are 43 to 135 times higher than values of this study. Endpoints observed for *L. variegatus* and *C. riparius* were more sensitive to TNT than a marine polychaete and an estuarine amphipod in 28-day sediment bioassays of GREEN *et al.* (1999) (LC_{50} for *Neanthes arenaceodentata* = 320 $mg\,kg^{-1}$, LC_{50} for *Leptocheirus plumulosus* = 203 $mg\,kg^{-1}$). Further, for *H. azteca* an EC_{50} (28 days) for the endpoint number of adults of 0.84 $mg\,kg^{-1}$ was reported by FIEDLER (personal communication), which indicates equal sensitivity of *H. azteca*, *L. variegatus*, and *C. riparius* to TNT.

A LOEC of 250 $mg\,kg^{-1}$ 3,4-DCA (based on nominal concentration) was determined for the endpoint dry weight in a 10-day sediment toxicity bioassay with *C. riparius* (NAYLOR & HOWCROFT, 1997). Whereas OETKEN *et al.* (2001) determined a LOEC of 3 $\mu g\,kg^{-1}$ for the endpoint EMT_{50}, but no effects on all other endpoints up the highest tested nominal concentration of 40 $mg\,kg^{-1}$. This is in good agreement with findings of RIBEIRO *et al.* (1999), who found a significantly decreased development rate down to the lowest tested concentration of 1.25 $mg\,l^{-1}$ (based on nominal, 41% were measured at day of exposure, three days after substance application) below lethal concentrations. All other endpoints were not affected at these concentrations. For the two highest concentrations of 10 and 20 $mg\,l^{-1}$ (4.13 and 9.69 $mg\,l^{-1}$measured at day 0) 100% mortality were observed after 5 days (RIBEIRO *et al.*, 1999). Effects at these concentrations should mainly be attributed to toxic effects via water-solved 3,4-DCA since the 48-hour LC_{50} of 6.1 $mg\,l^{-1}$ for first instar larvae is in the same range (see table 3.8 on page 28). For *H. azteca* an EC_{50} (28 days) for the endpoint biomass of adults of 0.57 $mg\,kg^{-1}$ was reported by FIEDLER (personal communication), which indicates equal sensitivity of *H. azteca*, *L. variegatus*, and *C. riparius* to 3,4-DCA.

No literature data were available for tests with DDT for *C. riparius* and *L. variegatus*. In 10-day DDT sediment bioassays with *H. azteca* (4 mm, plus feeding) with different total organic carbon (TOC) contents ranging from 3% to 10.5%, LC_{50} values ranged from 11.0 to 49.7 $mg\,kg^{-1}$ DDT (NEBEKER *et al.*, 1989), which is in the range of EC_{50} for *L. variegatus* of this 28-day study. *H. azteca* reacted less sensitively in systems with high TOC contents. In this study, *C. riparius* was 85 times more sensitive than *H. azteca* in the study of NEBEKER *et al.* (1989). Whereas, *H. azteca* reacted very sensitively in a 28-day study with sediment without external feeding, for which an EC_{50} of 0.01 $mg\,kg^{-1}$ for the endpoint number of adults was observed (FIEDLER, personal communication). The EC_{50} value was 13 and 4,890 times lower than EC_{50} values for *C. riparius* and *L. variegatus*, respectively.

The 14-day EC_{50} for *L. variegatus* was 2.2 $mg\,kg^{-1}$ for cadmium (CHAPMAN *et al.*, 1999) for spiked artificial sediment containing mainly sand with only 0.02% TOC. Whereas the calculated EC_{50} was 4.4 $mg\,kg^{-1}$ in sediments of this study with TOC of approximately 1%. Discrepancies are most likely explained by noting that metal ions were more bioavailable in artificial sediment with low organic carbon content. The amount of humic substances is higher in systems with higher TOC contents. Cadmium is adsorbed by humic substances (FU *et al.*, 1992) and thus less bioavailable at elevated humic substance concentrations (BORGMANN *et al.*, 1991). An important binding phase controlling interstitial water concentrations of the

metals is an extractable fraction of (iron) sulfides, known as acid-volatile sulfide (AVS). A number of studies have shown conclusively that when AVS concentrations exceed those of metal simultaneously extracted with the AVS, free metal concentrations in the interstitial water are low, and toxicity is not observed (ANKLEY *et al.*, 1996, and references therein). Further, higher amounts of AVS lower bioaccumulation and thus lead to toxic effects at higher concentrations (CHAPMAN *et al.*, 1999). A significant increase in mortality of *C. riparius* was observed in 16.2 $mg l^{-1}$ Cd in a bioassay with spiking via the water phase with shredded paper as substrate (POSTMA *et al.*, 1994). Effects may mainly be explained by exposure via the water phase, since a 48-hour LC_{50} of 4.8 $mg l^{-1}$ via water-only exposure was observed for first instar in this study (see table 3.8 on page 28). Further, in a study of SILDANCHANDRA & CRANE (2000) survival of *C. riparius* was significantly reduced at a measured concentration of 0.39 $mg kg^{-1}$. In an experiment using artificial sediments according to OECD guideline 218 with 5% organic matter, a LOEC on endpoint mortality of 0.3 $mg kg^{-1}$ and an LC_{50} value of 1.64 $mg kg^{-1}$ Cd were found (PEDINA, 2001). In a sediment toxicity test with quartz sand not containing organic matter with external feeding, VOGT *et al.* (in press) calculated an LC_{50} of 0.85 $mg kg^{-1}$ Cd. In this study, development rate was effected at concentrations as low as 0.12 $mg kg^{-1}$, whereas emergence and dry weight were significantly affected only at higher concentrations (12 $mg kg^{-1}$). Cadmium was found to delay emergence, which agrees with findings of other studies (WENTSEL *et al.*, 1978; PASCOE *et al.*, 1989; MCCAHON & PASCOE, 1991; POSTMA *et al.*, 1994; SILDANCHANDRA & CRANE, 2000; VOGT *et al.*, in press). The lower LC_{50} values reported by VOGT *et al.* (in press) are not surprising because of the high bioavailability of Cd due to the absence of organic matter; and thus, probably did not contain complexing agents such as AVS and humic substances. In a different study, cadmium LC_{50} values for *C. riparius* (10 day) and *H. azteca* (28 day) on bulk sediment, overlying water, and pore water were 39 and 33 $mg kg^{-1}$, 3.3 and 3.2 $\mu g l^{-1}$, and 18 and 33 $\mu g l^{-1}$, respectively (MILANI *et al.*, 2003). EC_{50} values on endpoint growth were 10 and 16 $mg kg^{-1}$ for *C. riparius* (10 day) and *H. azteca* (28 day) (MILANI *et al.*, 2003). In a 28-day study with *H. azteca*, an EC_{50} for the endpoint number of adults of 12.5 $mg kg^{-1}$ was observed (FIEDLER, personal communication). However, discrepancies within results of these investigations show that different test designs lead to different exposure conditions and thus different effect levels. Findings of this and other studies indicate that *L. variegatus* and *C. riparius* are more sensitive to cadmium than *H. azteca*.

No data in literature were available for B(a)P, PCP and 2,4-DCP tested on *L. variegatus* and *C. riparius*. For *H. azteca*, 28-day EC_{50} values of 2.26, 0.23, and < 0.25 $mg kg^{-1}$ were reported for B(a)P, PCP, and 2,4-DCP (FIEDLER, personal communication). *H. azteca* was more sensitive to B(a)P, PCP, and 2,4-DCP than *L. variegatus* and *C. riparius*.

3.4 Factors influencing bioavailability and toxicity in sediment toxicity tests

In addition to similarities, various differences among the observed effect concentrations of this study and literature data were reported in the previous section. These differences may have several reasons. Chemicals need to bioaccumulate in the organism to reach a body concentration at which some significant toxic effects occur. This concentration is described as critical body residue (CRB). The toxic effects of a chemical are related to CRB rather than solely to concentration in the environment (MCCARTY & MACKAY, 1993). When lethal body residues are reached, mortality is observed. LBR_{50} is the body residue at 50% mortality. The bioaccumulation and thus toxicity are dependent on (1) the impact of the contaminants characteristics, (2) environmental (sediment) characteristics, and (3) impact of biological factors on bioavailability.

3.4.1 Impact of contaminants characteristics on bioaccumulation

The octanol-water coefficient has often been used successfully to estimate bioconcentration potential of contaminants. Contaminant bioaccumulation behavior cannot solely be predicted by lipophilicity; other factors, such as contaminants steric and electrochemical characteristics, have an impact on bioaccumulation (LYYTIKÄINEN *et al.*, 2003, and references therein). Steric factors may influence contaminants desorption from sediment (LYYTIKÄINEN *et al.*, 2003). Bioavailability of a range of sediment associated nonpolar contaminants can be related to the fraction of contaminant rapidly desorbed (LAMOUREUX & BROWNAWELL, 1999; CORNELISSEN *et al.*, 2001). The findings of KUKKONEN *et al.* (2004) showed that bioavailability in freshwater benthic organisms *L. variegatus* and *Diporeia* spp. was best described by the fraction rapidly desorbed from several sediments for several PAHs and PCB congeners. Further, speciation of contaminant influences its availability.

3.4.2 Sediment characteristics and bioavailability

Several environmental characteristics affect toxicity of contaminants within sediments. Bioaccumulation is affected by organic carbon quantity and quality (like proportion of hydrophobic acids, functional groups, aromaticity, etc.) (KUKKONEN & OIKARI, 1991; HARKEY *et al.*, 1994), and the total sediment surface area that influence the number, type, and strengths of the bindings between sediment and the contaminant (LYYTIKÄINEN *et al.*, 2003). These factors influence bioavailability especially of lipophilic substances and metals. MÄENPÄÄ & KUKKONEN (2006) reported from a study with two surfactants (12C-LAS and 4-NP) that the more organic the sediment, the lower the bioaccumulation of chemical, which suggested that

a fraction of the chemicals was sequestered in a non-bioavailable pool of the sediment. Sorption of lipophilic substances increased with increasing organic carbon (OC) content of the sediments (WESTALL *et al.*, 1999, cited by MÄENPÄÄ & KUKKONEN, 2006). Further, it has been shown that contaminant binding affinity varies among different organic matter fractions in soil and sediment (KOHL & RICE, 1998; KUBICKI & APITZ, 1999). TOC of the sediment affects the amount of dissolved organic carbon (humic acid is one of its major components) in overlying and pore water. Dissolved organic carbon influences the bioavailability for anorganic and organic substances. It was observed that triorganotin compounds associated with Aldrich humic acid (AHA) are not bioavailable to *C. riparius* (LOOSER *et al.*, 2000). The presence of dissolved organic carbon increased the adsorption of 3,4-dichloroaniline (GONZALEZ-PRADAS *et al.*, 2005), whereas HEIM *et al.* (1995) reported that 3,4-DCA was primarily bound to the organic matter (the majority bound to the insoluble humin fraction) in a sediment with high clay and silt fraction and organic carbon (OC) content.

Further, it was shown that differences in distribution of the contaminants among particle size classes between the sediments do not completely correlate with the amount of OC in the size fraction (KUKKONEN *et al.*, 2003). Thus, the hypothesis that not only OC quality and quantity are important for contaminant binding, but that compositional dependence varies across each size class of natural particles (KUKKONEN *et al.*, 2003).

Other factors besides lipophilicity of the chemical play a role in the chemical distribution between organisms and the surrounding compartments. The contaminant distribution and movement are likely affected also by characteristics of the organisms and the sediments (MÄENPÄÄ & KUKKONEN, 2006). The chemical distribution among the different sediment fractions may vary and thus may affect the bioavailability of contaminants in general (KUKKONEN & LANDRUM, 1996). Studies of KUKKONEN *et al.* (2005) imply that compounds sorbed to plant-derived carbon are more bioavailable since this material is more likely ingested by benthic organisms providing a second exposure route.

It was shown by TILLMANN (2004) that inhibitions on Chironomids by triphenyltin were lower with higher amounts of small grain size in the sediment. Increasing the proportion of fine particles expands the total sediment surface area, and thus, the total number of adsorption, which may significantly lower the bioavailability of contaminants (LUOMA, 1989). Larval development of *C. riparius* (VOS *et al.*, 2002) and *C. tentans* (SIBLEY *et al.*, 1997) was affected in sediments with smaller grain size and limited food supply. Whereas, grain size has no effects in sediments with sufficient food supply (VOS *et al.*, 2002).

Changes in bioavailability of contaminants due to increased aging times can occur but may be chemical-specific and species-dependent (SCHULER & LYDY, 2001; SCHULER *et al.*, 2003). Changes in bioavailability of organic chemicals may partly be due to higher sequestration or entrapment within intraparticle micropores of the sediment over time (PIGNATELLO, 1990; STEINBERG *et al.*, 1987; ROBERTSON & ALEXANDER, 1998; SCHULER *et al.*, 2003). Increased sequestration or reduced desorption from sediment may result in lower interstitial water concentrations (SCHULER *et al.*, 2003). The assimilation efficiency of the sediment

sequestered chemicals may be limited during gut passage (LAMOUREUX & BROWNAWELL, 1999). Lethal toxicity to *Tubifex tubifex* decreased with aging time when exposed to TNT (CONDER *et al.*, 2004b). Because of rapid degradation of TNT, disappearance of degradation products, and partitioning to overlying water, only small amounts of the added nitroaromatic mass balance were associated with sediment (CONDER *et al.*, 2004b), which is in good agreement with the findings of this study.

Further, pH of sediment and overlying water influence the bioavailability for anorganic and organic substances. pH can alter the speciation of some chemicals. TBT at pH higher than its pka value of 6.51 exists mainly as hydroxy complex TBTOH or TBTCl, which is much more bioavailable than the cation that dominates at lower pH-values (FENT, 1996; LOOSER *et al.*, 1998). pH has an impact on the speciation of weak acids such as PCP. The amount of pentachlorophenol is decreasing with increasing pH, whereas the amount of pentachlorophenolate is increasing. An average pH of 8.3 was observed in the overlying water of the sediment toxicity tests of this study, indicating there was only small amount of pentachlorophenol (0.025%, according to the Henderson-Hasselbalch equation) and a large amount of the pentachlorophenolate in the water phase. The diffusion of this molecule through biological membranes is hindered, and therefore, the bioavailability is reduced.

For metals, the free metal ion is considered as the biologically most available species. However, the sensitivity of aquatic organisms to metals in water is dependent on the chemical characteristics of the water. Hardness, pH, chemical complexing agents, and particulate matter all influence toxicity (BORGMANN, 1983). Alkalinity and salinity can affect the speciation of metal ions by increasing ion-pair formation, thus decreasing free metal ion concentration. Complexing agents, such as humic acids, sediment extracts, and AVS bind to metals in solution and reduce free metal ions available for uptake.

Water hardness affects accumulation and thus toxicity. Acute *Ceriodaphnia dubia* median lethal concentrations for Ni increased with increasing water hardness (KEITHLY *et al.*, 2004). The same study reported that chronic toxicity was less dependent on hardness than was acute toxicity. Water hardness had only minor effects on bioaccumulation and toxicity towards *H. azteca* (BORGMANN *et al.*, 1991). Nevertheless, calcium ions have been shown to inhibit Cd bioaccumulation in *H. azteca* and to decrease toxicity (STEPHENSON & MACKIE, 1989).

Cd and Zn uptake in larvae of *C. riparius* was increased with increasing pH from 5 to 8 (BERVOETS & BLUST, 2000). SCHUBAUER-BERIGAN *et al.* (1993) found an increase of toxicity of Cd and Zn with increasing water pH for aquatic invertebrates. It is hypothesized in literature that free metal ions are in competition with hydrogen ion at the membrane level and therefore restrict uptake under acid conditions (CAMPBELL & STOKES, 1985; HARE & TESSIER, 1996; CROTEAU *et al.*, 1998; BERVOETS & BLUST, 2000). However, at higher pH (increase from pH 9 to 10), a decrease in metal uptake of larvae was observed but remained high overall (BERVOETS & BLUST, 2000). Authors further postulate that an increase in pH alters the metal uptake process by decreasing the protonation of the binding sites. In the same study, acclimation to different pH remarkably affected accumulation of Cd and Zn by

the midge larvae. It was postulated that pH has an effect not only on metal speciation or protonation of the binding sites, but also alters the physiological condition of an organism, and thus indirectly affects metal uptake.

As for lipophilic organic contaminants, for metals similar effects of humic substances were described by BORGMANN *et al.* (1991) and FU *et al.* (1992). TOC was proven to be an important sediment factor for the bioavailability of Cd (BERVOETS *et al.*, 2004) and other metals (BERVOETS *et al.*, 1997, 2004; TESSIER *et al.*, 1984) to benthic organisms.

The influence of AVS on the bioavailability and toxicity of divalent metals in sediments through the formation of insoluble metal sulfide complexes has been demonstrated (ANKLEY *et al.*, 1991; CARLSON *et al.*, 1991; DEWITT *et al.*, 1996; HANSEN *et al.*, 1996, and others). In a recent study with *Neanthes arenaceodentata*, it was shown that not only Cd and Zn tissue concentrations were significantly higher in sediments with low AVS concentration at a given simultaneously extracted metal concentration, but also toxicity (both mortality and reduced growth rate) was observed due to increased dissolved metal concentrations in overlying water (LEE & LEE, 2005). Further, it was shown that redoxpotential has a high impact on the bioavailability of metals (GAMBRELL *et al.*, 1991; SUEDEL & RODGERS, 1994). In sediments with reducing characteristics, AVS have a significant impact on the regulation of cadmium toxicity (DITORO *et al.*, 1990).

3.4.3 Impact of biological factors on bioavailability

Biological factors (such as feeding, intestinal absorption, surfactant activity of digestive fluid, and elimination rate) are probably essential in determining the magnitude of bioaccumulation (LYYTIKÄINEN *et al.*, 2003). LEPPÄNEN & KUKKONEN (2006) reported that nonfeeding *L. variegatus* accumulated less contaminants (B(a)P, Pyrene and 3,4,3´,4´-tetrachlorobiphenyl) than feeding ones, thus showing that different bioavailable fractions were experienced. Besides feeding, differences in animal activity may also modify bioaccumulation (LEPPÄNEN & KUKKONEN, 2006). Also, gut residence time, which varies for different sediments, may influence bioavailability according to his study. Further, it was reported that diagenetically younger organic material (plant pigments, lipids, and lignin) may be more susceptible to assimilation and thus, the release of contaminants (KUKKONEN *et al.*, 2003, 2005; LEPPÄNEN & KUKKONEN, 2006).

The two benthic organisms used in this study differ in feeding behavior, which may alter exposure pathways and bioavailability. Though both ingest fine particles, *L. variegatus* is considered a general feeder ingesting any and all particles small enough to fit in its mouth (KUKKONEN *et al.*, 2004), while *C. riparius* is considered to feed selectively on fine organic material. Different feeding habits of species influence their exposure to contaminants bound to organic carbon. Differences in the structural and functional organization of the organisms, which among others determine the route of exposure, are at least equally important causes of

variability in contaminant availability, accumulation, and toxicity. Differences in the biotransformation and metabolizing ability of *L. variegatus* and *C. riparius* may explain differences in the observed sensitivity.

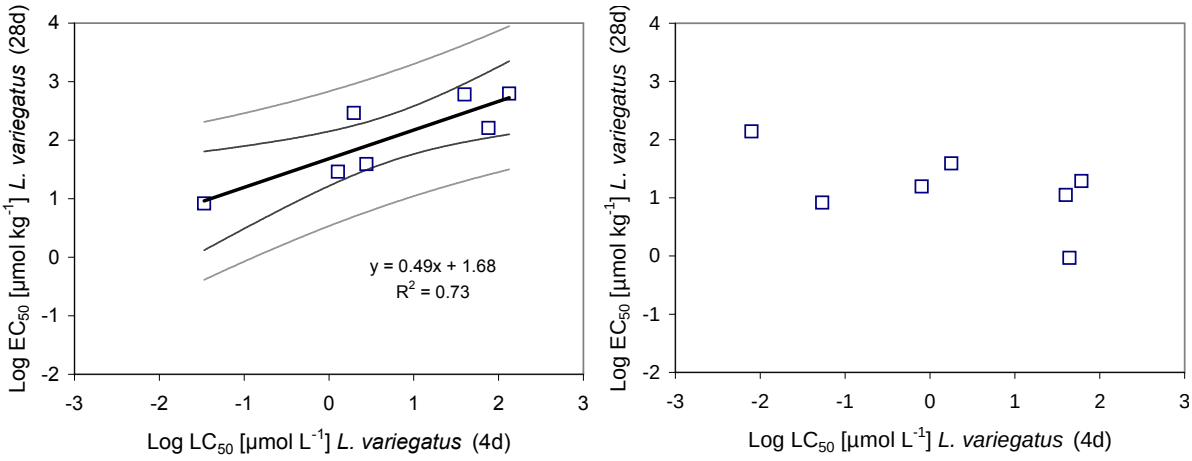
Differences in sensitivity were reported among organisms of the same species and the same chemical when tested in several laboratories. The largest observed difference between minimum and maximum LC_{50} and LOEC value within an international ring test for validation of a sediment toxicity test with *L. variegatus* and PCP were 4.3 and 23.5, respectively (EGELER *et al.*, 2005). Further, differences in species sensitivities of *C. riparius* of up to a factor of 10 were observed among genetically differing strains from different laboratory cultures and natural populations in sediment toxicity tests for a certain contaminant (VOGT, personal communication). It was shown that bioaccumulation of sediment associated-contaminants was clearly higher for feeding versus nonfeeding worms (LEPPÄNEN & KUKKONEN, 2006). Sediment ingestion associated with animal activity such as burrowing can expose animals to the larger bioavailable fraction. Dissolved chemical is subsequently increased and thus made bioavailable with the digestion of organic matter in the gut (LEPPÄNEN & KUKKONEN, 2006).

3.5 Correlation and comparative discussion of acute and sediment toxicity data

The following sections discuss the fourth main aspect of this study: whether the prediction of sediment toxicity data from acute toxicity data is possible.

3.5.1 *L. variegatus* - correlation of acute with sediment toxicity data

Correlation of both nominal and effective data of 96-hour LC_{50} data of *L. variegatus* of acute toxicity tests with 28-day EC_{50} data of *L. variegatus* sediment toxicity tests are shown in figure 3.50. Because the data were not bivariate normally distributed, the Spearman's rank correlation test was used for correlation analysis. Data were not transformed before the correlation analysis. Acute 96-hour LC_{50} data significantly correlate with 28-day EC_{50} data of the sediment toxicity tests ($\rho = 0.82$, $p \leq 0.05$) based on nominal concentrations, whereas no significant correlation was observed between data based on effective concentrations ($\rho = -0.32$, $p = 0.50$). A prediction of sediment toxicity data for *L. variegatus* would not be possible from acute toxicity data based on effective concentrations. For data based on nominal concentrations, linear regression analysis was performed because of significant correlation between the data and the fact that conditions were fulfilled for logarithmized data to perform a linear regression analysis (figure 3.50(a)). However, the usage of the significant correlation based on



(a) based on nominal concentration (b) based on effective concentration

Figure 3.50: Correlation of effective 96-hour LC_{50} of *L. variegatus* with 28 d EC_{50} of *L. variegatus* sediment toxicity tests, grey line = 95% confidence (line), light grey line = 95% confidence (data)

nominal concentrations is not meaningful because prediction should be done for measured concentrations.

Observed versus expected EC_{50} data based on the regression model of data based on nominal concentrations is shown in figure 3.51. The uncertainty factors for the eight tested substances were lower than 10. The largest difference in observed versus estimated effect data showed DDT with a factor of 4, which was an overestimation of toxicity. DDT is a substance

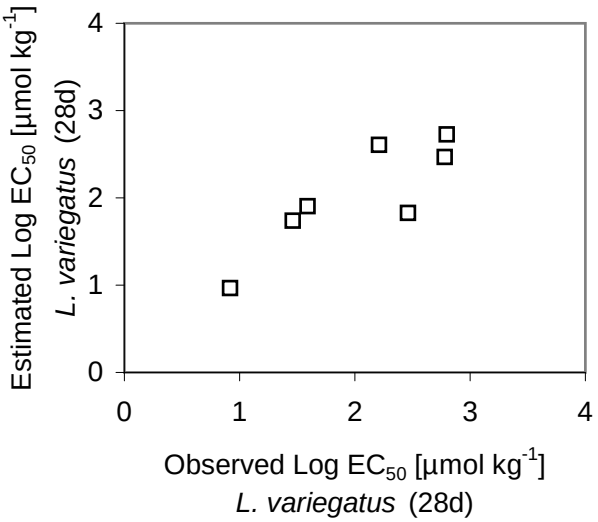
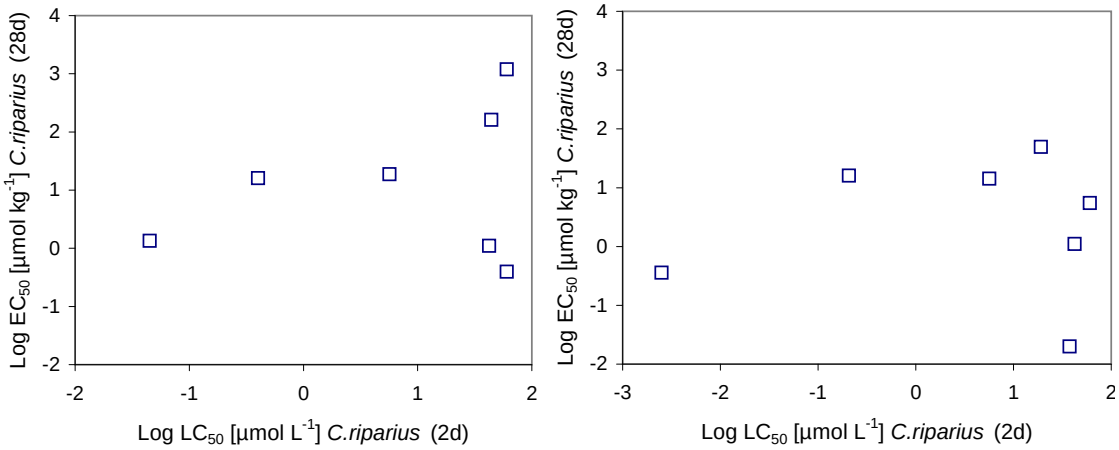


Figure 3.51: Observed versus predicted EC_{50} of *L. variegatus* 28 d sediment toxicity tests using prediction from acute 96-hour toxicity test, based on nominal concentrations (see model in figure 3.50 a)

with a log K_{ow} higher than 5. It needs to be pointed out that values for B(a)P were not available, since it was not possible to show any effects in the acute toxicity test up to 2 $mg l^{-1}$, which is far above water solubility level. Statements based on nominal concentrations do not reflect natural concentrations. Thus, measured (real) concentrations need to be used for data comparison.

3.5.2 *C. riparius* - correlation of acute with sediment toxicity data

Correlation of both nominal and effective data of 48-hour LC_{50} of acute toxicity tests with 28-day EC_{50} of *C. riparius* sediment toxicity tests are shown in figure 3.52. Because the



(a) based on nominal concentration (b) based on effective concentration

Figure 3.52: Correlation of 48-hour LC_{50} of *C. riparius* with 28 d EC_{50} of *C. riparius* sediment toxicity tests

data were not bivariate normally distributed, the Spearman's rank correlation test was used for correlation analysis. Data were not transformed before the correlation analysis. For both nominal and effective, 48-hour LC_{50} data are not significantly correlated with 28-day EC_{50} data (based on nominal data: $\rho = 0.25$, $p = 0.59$, based on effective data: $\rho = -0.14$, $p = 0.78$). Thus, a prediction of sediment toxicity data for *C. riparius* would not be possible from acute toxicity data. The absence of significant correlation is mainly due to the values of cadmium and 3,4-DCA, which showed relatively low toxicity in the acute 48-hour toxicity test. Whereas in the sediment toxicity tests the lowest EC_X/ LOEC values of the tested substances were observed.

3.6 Comparative discussion of partition coefficients for sediment-pore and -overlying water partitioning

Partition coefficients (k_p) for sediment pore water and sediment overlying water partitioning were calculated from analytically measured concentrations in bulk, pore water, and overlying water.

3.6.1 Tributyltinchloride

Mean partition coefficients of the two sediment toxicity tests were determined from analyzed samples at the start and end of the exposure. Calculated partition coefficients for TBT were 1575 l/kg for sediment-pore water partitioning and 2490 l/kg for sediment-overlying water partitioning over the exposure period of the three sediment toxicity assays. These are in good agreement with values of several investigations (DAY *et al.*, 1998; FENT, 1996, and references therein). The corresponding partition coefficient values based on organic carbon (organic carbon content ranged from 0.75% for *L. variegatus* to 1% for *C. riparius*) are 122,250 l/kg and 201,850 l/kg, respectively. These values may be comparable to values of 25,100 (MEADOR *et al.*, 1997), 37,000 (DAY *et al.*, 1998) for the sediment-pore water partitioning, and 107,000 (DAY *et al.*, 1998) for the sediment-overlying water partitioning of TBT determined by others. The usage of the mean of the two tests may be questionable since high variations were observed among concentrations used in one single test system, sampling dates, and different test systems. Mean concentration in pore water is higher by a factor of 2 than in overlying water. However, high variations were observed with smaller differences for tests with *C. riparius* and larger differences for *L. variegatus*. Partition coefficients for sediment water were highest for *C. riparius* (2040 l/kg for sediment-pore water partitioning) and lowest for tests with *L. variegatus* with 38 l/kg for sediment-pore water partitioning, respectively. Water-to-sediment ratio was higher in experiments with *L. variegatus* compared to *C. riparius*, resulting in relatively high volumes above thin sediment layers. The process of water exchange between overlying and pore water of the whole sediment is increased. Variations among test systems for partition coefficients were observed. Partition coefficients decreased for *C. riparius* over the course of the experiment. Whereas for *L. variegatus*, the situation was not clear. Sediment-overlying water partition coefficients increased, whereas sediment-pore water partition coefficients were the same at the start and end of the exposure.

3.6.2 2,4,6-trinitrotoluene

Partition coefficients were determinable only for the highest TNT concentration tested with *L. variegatus*. All other water concentrations were below the limit of detection (4 $\mu g l^{-1}$). Partition coefficients were fairly low with k_p= 3.2 l/kg for sediment overlying water partitioning

and k_p= 10.9 l/kg for sediment pore water partitioning for sample t0 for the highest concentration of the *L. variegatus* test. Reasons for low partition coefficients are the relatively low log K_{ow} of 1.6 and relatively high water solubility of 0.13 gl^{-1} at 20 °C.

3.6.3 Pentachlorophenol

Mean sediment-pore water partition coefficients were nearly equal for the two sediment toxicity tests (20 l/kg for *L. variegatus* and to 23 l/kg for *C. riparius*). Mean sediment-overlying water partition coefficients were 19 l/kg for *C. riparius*. A higher mean value of 34 l/kg was observed for *L. variegatus*. Sediment-pore water partition coefficients equaled sediment-overlying water partition coefficients in the *C. riparius* sediment toxicity test. In the sediment toxicity test with *L. variegatus*, pore water concentrations were about twice as high as overlying water concentrations. Sediment composition of *C. riparius* tests was quite different compared to that used for *L. variegatus*. The sediment used in the test with *L. variegatus* contained peat, kaolin, and fine quartz sand, which provided sediments of very small grain size, resulting in a higher total surface area. Exchange processes between overlying water and sediment may therefore have been reduced. These differences may explain the relatively lower concentrations in overlying water compared to the test with *C. riparius*.

3.6.4 2,4-Dichlorophenol

Mean sediment-pore water and -overlying water partition coefficients were 2.5 l/kg and 5.3 l/kg, respectively, for both sediment toxicity tests. Reasons for the relatively low partition coefficients when compared to tests with other chemicals are the relatively low log K_{ow} of 2.8 and the fairly high water solubility of 4.5 gl^{-1} (20 °C). The ratio of sediment-pore water and -overlying water concentrations increases with increasing concentrations for both *L. variegatus* and *C. riparius*, with the exception of the ratio of sediment-overlying water concentrations for *C. riparius*, where no clear trend was observed. Mean relative pore water concentrations of the lowest analyzed concentration of *L. variegatus*/ *C. riparius* were 2.9/ 2.1 times lower than in the highest concentration analyzed. Mean relative overlying water concentration of the lowest concentration for *L. variegatus* was 1.6 times lower than for highest concentration. Relative concentrations in pore water of *C. riparius* sediment bioassay are 2.6 times higher than in overlying water. No difference was observed in the test with *L. variegatus*. Higher partition coefficients for sediment water partitioning were observed for the sediment toxicity test with *C. riparius* when compared to the sediment toxicity test with *L. variegatus*.

3.6.5 3,4-Dichloroaniline

Partition coefficients for 3,4-DCA were calculated from the report of OETKEN *et al.* (2001). Calculated partition coefficients for 3,4-DCA were 0.2 l/kg for sediment-pore water parti-

tioning and 2.9 l/kg for sediment-overlying water partitioning over the exposure period of *C. riparius*. For *L. variegatus*, values were 0.4 l/kg and 3.3 l/kg, respectively. Effective concentrations on bulk were only 1.4% of nominal concentrations for the tests with *L. variegatus* and *C. riparius*. The concentrations in overlying water were 18 and 8 times lower than in pore water of tests with *C. riparius* and *L. variegatus*, respectively. Partition coefficients for sediment pore/ overlying water partitioning decreased with increasing concentrations for bioassays with *L. variegatus* and *C. riparius*. No clear trend was observed for differences in ratios between the start and end of the exposure. The relatively low partition coefficients were unexpected, but may be due to the small amounts extractable (only 1.4%) from the sediment.

3.6.6 Benzo-[a]-pyrene

High partition coefficients for sediment water partitioning were observed for B(a)P. The calculated mean partition coefficient for sediment pore water partitioning was 811 l/kg for the bioassays of the two invertebrates. The mean ratio was 355,632 l/kg for sediment overlying water partitioning, respectively. Measured pore water concentrations were 74 (for the test with *L. variegatus*) and 605 (for the test with *C. riparius*) times higher than overlying water concentrations. High variation among different test systems was observed. Lower ratios were observed for the test with *L. variegatus* compared to *C. riparius*, which may be explained by a thinner sediment layer and high water-to-sediment ratio (8:1) resulting in a higher exchange between overlying water and sediment. Ratios for sediment pore water partitioning were 508 and 1114 l/kg for *L. variegatus* and *C. riparius*, respectively. Ratios for sediment overlying water partitioning were 37,596 and 673,668 l/kg, respectively. Overall, ratios increased with increasing concentration. There are no clear trends for changes between sampling dates. For *C. riparius*, both partition coefficients increased over time. For *L. variegatus*, ratios of sediment overlying water partitioning decreased while that for sediment pore water partitioning were nearly the same with increasing exposure time.

3.6.7 4,4-Dichlorodiphenyltrichloroethan

High partition coefficients for sediment water partitioning were observed for DDT. The calculated mean partition coefficient for sediment pore water partitioning was 7753 l/kg for the bioassays of the two invertebrates. The mean ratio was 65,223 l/kg for sediment overlying water partitioning. Measured pore water concentrations were 3 (for tests with *C. riparius*) to 11 (for tests with *L. variegatus*) times higher than overlying water concentrations. The highest partition coefficients for sediment water partitioning were observed for the test with *L. variegatus*. Only for the highest measured concentrations, pore and overlying water concentrations were above the limit of quantification. Therefore, a general conclusion on the variation in change of ratios with increasing concentrations and exposure time cannot be made. For the

test with *L. variegatus*, where pore and overlying water concentrations were detectable in two concentrations, partition coefficients were higher for the higher concentration.

3.6.8 Cadmiumchloride

The calculated mean partition coefficient for sediment pore water partitioning was 4.1 l/kg for the bioassays of the two invertebrates. The mean ratio was 130 l/kg for sediment overlying water partitioning. Measured pore water concentrations were 17.5 (for tests with *L. variegatus*) and 105 (for tests with *C. riparius*) times higher than overlying water concentrations. High variation among the two test systems was observed. Ratios for sediment pore water partitioning were 1.3 and 6.8 l/kg for *C. riparius* and *L. variegatus*, and thus different by a factor of 5. Ratios for sediment overlying water partitioning were 141 and 119 l/kg respectively and thus in good agreement. Ratios of sediment pore water partitioning increased with increasing concentration, while ratios for sediment overlying water partitioning decreased with increasing concentrations. There are no clear trends for changes between sampling dates.

3.6.9 Summary of partition coefficients of sediment-water partitioning

Overall, the highest partition coefficients for sediment water partitioning were observed for B(a)P (356,000 l/kg), DDT (65,000 l/kg), and TBT (2500 l/kg), which are the chemicals with high log K_{ow}. From equilibrium partition theory based on log K_{ow}, relatively less DDT concentration in water than B(a)P in water would be expected. This was not the case. One explanation may be that B(a)P is known to be highly sequestered in organic matter, resulting in very slow desorption. Whereas, the lowest were observed for chemicals with relatively lower log K_{ow} and higher water solubility. In general, contaminant pore water concentrations were higher than overlying water concentrations with the exception of the PCP test with *C. riparius*, the 2,4-DCP test with *L. variegatus*, and the TNT test with *L. variegatus*. For the TNT test, only the highest concentration was evaluable. All other concentrations of TNT tests with *C. riparius* and *L. variegatus* were below the limit of detection. The largest difference between pore and overlying water concentration was observed for B(a)P with a factor of 605. For three of seven chemicals, higher partition coefficients for sediment water partitioning were observed for sediment toxicity tests with *C. riparius* than with *L. variegatus*, resulting in relatively lower water concentrations for *C. riparius*. In three cases, partition coefficients were equal for both test systems and in one case, sediment water partition coefficients were higher for tests with *L. variegatus*. The on average higher partition coefficients observed for tests with *C. riparius* are partly due to the slightly higher OC content compared to tests with *L. variegatus*. Further, exchange processes between overlying water and sediment are slower in test systems with thick sediment layers like the one used for *C. riparius* compared to thinner ones as used for *L. variegatus*. Similar observations were made in sediment toxicity studies with

V. americana where sediment layers were relatively thick as well (FIEDLER *et al.*, in prep.). Further, bioturbation affects concentrations in overlying water. It was shown that higher bioturbation increases overlying water concentrations of B(a)P (CLEMENTS *et al.*, 1994). It is not clear from this study, whether the activity of the different organisms leads to differences in bioturbation.

3.7 Exposure effect assessment for sediment toxicity tests

When benthic organisms are exposed to contaminated sediment, several exposure routes are important. Organisms accumulate contaminants via their solved form in overlying water, pore water, and by ingestion of substance adsorbed to the sediment. The latter two are especially important for benthic organisms. As outlined above, desorption of the contaminant from sediment particles into pore water or when ingested into the intestinal fluid drives bioaccumulation (LAMOUREUX & BROWNAWELL, 1999; CORNELISSEN *et al.*, 2001; TEN HULSCHER *et al.*, 2003; KUKKONEN *et al.*, 2004). This occurs regardless of the route of exposure prior absorption across a membrane. The intestinal fluid may be more efficient, enhancing either the desorption rate or the extent of desorption (VOPARIL & MAYER, 2000). The ingestion of organic matter in the gut subsequently increases dissolved chemical. To answer the question of which exposure route (overlying water, pore water, or sediment ingestion) drives chronic toxicity in sediment toxicity tests (the fifth main aspect of this study), substance concentrations in sediment, pore water, and overlying water were analytically determined. Following, acute toxicity of the substance to the test organism via the water phase were compared with pore and overlying water concentrations measured in effect concentrations of the chronic 28-day sediment toxicity studies. If acute LC_{50} is lower or equal to the measured concentrations at effect concentrations of the sediment toxicity test, then the main exposure route is likely via pore/ overlying water-solved substance. Whereas, if LC_{50} is higher, the pathway via ingested sediment may be of more importance. The exposure effect assessment was done in great detail for PCP, while a shorter description is given for all other substances. The following comparisons for finding the main exposure route can only give an indication of the true exposure situation.

True exposure of the organisms cannot be assessed solely by measuring contaminant concentrations surrounding the organism (such as pore/ overlying water and bulk sediment). The concentrations in the animal better reflect the true exposure situation (MCCARTY & MACKAY, 1993). Inner concentrations were not measured within the sediment toxicity tests of this study. However, BARRON *et al.* (2002) and MÄENPÄÄ & KUKKONEN (2006) reported high variability of critical body residues (CBR) among nonpolar narcotics, which is not in accordance with the hypothesis of the CBR approach. BARRON *et al.* (2002) evaluated CBR-values for different chemical classes and mode of action categories and concluded that a broad application of the CBR concept across chemical classes is not supported due to high variability. The authors

further note that the variability observed in tissue residues between chemicals within a given mode-of-action class appears to be generally of the same order of magnitude as the variability of aqueous measures of toxicity such as LC_{50} values. Nevertheless, MÄENPÄÄ & KUKKONEN (2006) note that if only LC_{50} values were considered, no information about true exposure (i.e., body residue) would have been gathered. Even though MÄENPÄÄ & KUKKONEN (2006) state that no prediction of body residue (i.e., internal exposure) can be made by only measuring, for example, the sediment chemical concentration, the method of comparing acute toxicity data with interstitial and overlying water concentrations measured for effect concentration indicates the main exposure route of the organisms towards a specific contaminant.

3.7.1 Pentachlorophenol

3.7.1.1 Sediment toxicity test with *L. variegatus*

3.7.1.1.1 Results - Acute toxicity test with *L. variegatus* and PCP An LC_{50} (96 h) of 0.34 $mg\,l^{-1}$ (95% confidence limits: 0.31, 0.37) was calculated using the Trimmed Spearman Karber method.

3.7.1.1.2 Results - 28-day Sediment toxicity test with *L. variegatus* and PCP The effect data are summarized in table 3.20.

Table 3.20: Effect data of 28 d *L. variegatus* sediment toxicity test with pentachlorophenol (nominal concentrations, in $mg\,kg^{-1}$)

Chemical	Endpoint	NOEC/LOEC	EC_{10}	EC_{50}
pentachlorophenol	reproduction	6.25 / 31.25	6.2	13.1
	biomass (dw)	6.25 / 31.25	1	7.7

A NOEC of 6.25 $mg\,kg^{-1}$ dw and a LOEC of 31.25 $mg\,kg^{-1}$ were calculated for the endpoints reproduction and biomass (as dry weight).

3.7.1.1.3 Results - analytical measurements in each compartment Table 3.21 shows the analytical measurements of each compartment. Samples of the lowest, median, and highest concentration were analyzed on sampling day 0 (date of exposure of test organisms) and on day 28 (end of the test).

Table 3.22 shows the calculated concentrations for the concentration of 6.25 $mg\,kg^{-1}$ (= NOEC), which was not analytically measured. Concentrations were calculated for each compartment using the average partitioning from the lowest, median, and highest concentration. The calculated concentrations of PCP are: sediment, 4.89 $mg\,kg^{-1}$; overlying water, 0.13 $mg\,l^{-1}$; and pore water, 0.23 $mg\,l^{-1}$.

Table 3.21: PCP concentrations of sediment, overlying and pore water for sediment toxicity test with *L. variegatus*

nominal ($mg\,kg^{-1}$)	sampling date	replicate	Sediment [$mg\,kg^{-1}$] dw	OW [$mg\,l^{-1}$]	PW [$mg\,l^{-1}$]	balance [$mg\,kg^{-1}$] dw
0.05	0	1	0.04	0.00087	0.00150	0.04
	0	2		0.00087	0.00133	
1.25	0	1	0.93	0.024	0.043	1.0
	0	2		0.025	0.041	
31.25	0	1	25.1	0.97	2.04	28.23
	0	2		0.94	1.89	
0.05	28	1	<0.03	0.00069	0.0016	0.02
	28	2	<0.03	0.00076	<0.00052	0.02
1.25	28	1	0.26	0.006	0.015	0.28
	28	2		0.012	0.018	
31.25	28	1	17.04	0.90	1.46	19.52
	28	2		0.91	1.55	

Table 3.22: Calculated sediment, overlying and pore water concentrations for the NOEC of sediment toxicity test with *L. variegatus*

nominal ($mg\,kg^{-1}$)	sampling date	replicate	Sediment [$mg\,kg^{-1}$] dw	OW [$mg\,l^{-1}$]	PW [$mg\,l^{-1}$]	balance [$mg\,kg^{-1}$] dw
Estimation of concentration in the not measured concentration = NOEC						
6.25	0		4.89	0.13	0.23	

3.7.1.1.4 Comparison of acute toxicity data with analytical measurements

The concentration of PCP at the NOEC and LOEC are of interest for the assessment of the effects. A bulk sediment concentration of 25.1 $mg\,kg^{-1}$ dw was measured in the highest concentration at sampling day 0. A pore water concentration of 1.96 $mg\,l^{-1}$ (mean of 2 analyzed replicates) and an overlying water concentration of 0.95 $mg\,l^{-1}$ (mean of 2 analyzed replicates) was measured at sampling day 0. A balance of 28.23 $mg\,kg^{-1}$ dw was calculated for day 0.

A bulk sediment concentration of 17.04 $mg\,kg^{-1}$ was measured in the highest concentration at sampling day 28. A pore water concentration of 1.5 $mg\,l^{-1}$ (mean of 2 analyzed replicates) and an overlying water concentration of 0.90 $mg\,l^{-1}$ (mean of 2 analyzed replicates) was measured at sampling day 28. A balance of 19.52 $mg\,kg^{-1}$ was calculated for day 28.

Measured concentrations in overlying and pore water at the start and end of the exposure exceeded the LC_{50} (96 h) of 0.34 $mg\,kg^{-1}$ by a factor higher than 2.6-5.8. Thus, it can be assumed that the toxic effect was due to the concentration of PCP found in overlying and pore water. These findings are supported by the observation that worms did not burrow into the sediment of the highest concentration (30 $mg\,kg^{-1}$) and were found dead after 4 to 6 days due to lysis. Thus, acute toxicity via water-solved PCP was obvious.

The estimated concentrations at the LOEC of 6.25 $mg\,kg^{-1}$ was 0.13 $mg\,l^{-1}$ in overlying and 0.23 $mg\,l^{-1}$ in pore water (see table 3). This concentration is almost equal to the LC_{50} (96 h); therefore, effects would be expected.

3.7.1.1.5 Summary of the exposure effect assessment on the example of the *L. variegatus* sediment toxicity test with PCP Analysis of pore and overlying water of the LOEC of 28-day *L. variegatus* sediment toxicity test with pentachlorophenol showed concentrations higher than the calculated LC_{50} (96 h) of the acute toxicity test (exposure via the water phase). Consequently, the observed effects are caused by exposure via the water phase. At a concentration of 4.89 $mg\,kg^{-1}$ PCP, no effects were found in the 28-day test.

3.7.1.2 Sediment toxicity test with *C. riparius*

3.7.1.2.1 Results - Acute toxicity test with *C. riparius* and PCP An LC_{50} (48 h) of 1.5 $mg\,l^{-1}$ (95% confidence limits: 1.17, 1.92) was calculated using probit analysis.

3.7.1.2.2 Results - 28-day Sediment toxicity test with *C. riparius* and PCP A NOEC of 0.4 $mg\,kg^{-1}$ and a LOEC of 3.8 $mg\,kg^{-1}$ were calculated for the endpoints individual dry weight of females and males. The lowest EC_{50} of 34.4 $mg\,kg^{-1}$ was derived for the endpoint total dry weight of males.

3.7.1.2.3 Results - analytical measurements in each compartment The analytical measurements of each compartment are summarized in table 3.9 on page 36. Samples of the treatments 0.5, 5, and 500 $mg\,kg^{-1}$ were analyzed on sampling day 0 (date of exposure of test organisms) and on day 28 (end of the test). The concentration of PCP at the NOEC and LOEC are of interest for the assessment of the effects. Bulk sediment concentrations of 0.42 $mg\,kg^{-1}$ on sampling day 0 and 0.37 on day 28 were measured in the nominal concentration of 0.5 $mg\,kg^{-1}$, which was the NOEC concentration. Pore water concentrations of 0.021/ 0.01 $mg\,l^{-1}$ on day 0/ 28 and an overlying water concentration of 0.015/ 0.019 $mg\,l^{-1}$ were measured, respectively. Bulk sediment concentrations of 3.8 $mg\,kg^{-1}$ on sampling day 0 and 28 were measured in the nominal concentration of 5 $mg\,kg^{-1}$, which was the LOEC concentration. Pore water concentrations of 0.07/ 0.25 $mg\,l^{-1}$ on day 0/ 28 and an overlying water concentration of 0.20/ 0.14 $mg\,l^{-1}$ were measured, respectively.

3.7.1.2.4 Comparison of acute toxicity data with analytical measurements Measured concentrations in overlying and pore water at the start and end of the exposure did neither exceed the LC_{10} (48 hour) of 1.0 $mg\,l^{-1}$ nor the LC_{50} (48 hour) of 1.5 $mg\,l^{-1}$, which were higher by a factor of 4 to 7 than the highest measured overlying/ pore water concentration. Thus, it can be assumed that acute toxic effects via the water phase can be excluded.

3.7.1.3 Summary of exposure effect assessment for sediment toxicity tests with PCP

Measured concentrations in the LOEC of the sediment toxicity tests were equal to the LC_{50} of the acute toxicity tests for *L. variegatus*. Thus, the main exposure and resulting toxicity must have occurred via water-solved PCP, which is supported by the observation that the worms of highest concentration (LOEC) did not burrow into the sediment and died within 6 days. For *C. riparius*, measured concentrations in the LOEC of the sediment toxicity tests were 7 times lower than the LC_{50} of the acute toxicity tests. Thus, the acute toxicity via the water phase at the start of the exposure can be excluded. The main exposure likely took place via sediment and particle-bound PCP. Results indicate that dietary uptake of PCP contributed the main part to toxicity.

Pentachlorophenol is a weak acid with a pka value of 4.7 (25 °C). pH values of the water phase in the acute toxicity tests were on average 7.5 for tests with *L. variegatus* and 7.2 for tests with *C. riparius*. At pH values of 8.2 for *L. variegatus* and 8.4 for *C. riparius* (mean values of all measured replicates) in the overlying water of the sediment toxicity test, only small amounts of pentachlorophenol (0.03 and 0.02%, respectively, according to Henderson-Hasselbalch equation) were present, with large amounts of the pentachlorophenolate in the water phase. The diffusion of this molecule through biological membranes is hindered, and therefore, the bioavailability is reduced. The amount of nonionic PCP was small as well in the water phase of the acute toxicity tests (0.1% for *L. variegatus* and 0.32% for *C. riparius*).

3.7.2 2,4-Dichlorophenol

The analytical measurements of each compartment of selected concentrations are summarized in table 3.10 on page 36.

3.7.2.1 Sediment toxicity test with *L. variegatus*

NOEC / LOEC values were 1.3 / 5.4 $mg\,kg^{-1}$ for all observed endpoints. The lowest EC_{50} of 3.2 $mg\,kg^{-1}$ was derived for the endpoint total number of worms. The highest measured concentrations of overlying and/ or pore water samples of the lowest 28-day LOEC at the start and end of the exposure were equal to the LC_{50} (96 h) of 9.9 $mg\,l^{-1}$ (95% confidence limits: 8.7, 11.4 $mg\,l^{-1}$). The highest value of 8.5 $mg\,l^{-1}$ was measured in pore water samples at the start of the exposure period. Thus, it can be assumed that acute toxic effects have occurred via the water phase.

3.7.2.2 Sediment toxicity test with *C. riparius*

The lowest NOEC / LOEC values were 5.2 / 10.4 $mg\,kg^{-1}$. The lowest EC_{50} of 8.2 $mg\,kg^{-1}$ was derived for the endpoint emergence. The highest measured concentrations of overlying and/ or pore water samples of the lowest 28-day LOEC at the start and end of the exposure exceeded the LC_{50} (48 hour) of 3.1 $mg\,l^{-1}$. The highest value of 7.2 $mg\,l^{-1}$ measured in pore water samples at the start of the exposure period was higher by a factor of 2.3 than the LC_{50} (48 hour). Thus, it can be assumed that acute toxic effects via the water phase cannot be excluded.

3.7.2.3 Summary of exposure effect assessment of sediment toxicity tests with 2,4-DCP

For *C. riparius*, measured concentrations in the LOEC of the sediment toxicity test were higher by a factor of 2.3 than the LC_{50} of the acute toxicity test. Concentrations were equal for *L. variegatus*. Thus, main toxicity via the water phase must have occurred. It is very likely that the main exposure for the two organisms took place via water-solved 2,4-DCP.

It should be noted that the speciation of 2,4-dichlorodiethyl is dependent upon pH because it is a weak acid with a pka value of 7.89 (25 °C). pH of the water phase ranged from 7.2 to 7.7 for the different acute toxicity tests. At pH values ranging from 8.1 to 8.4 (mean value of all measured replicates) in the overlying water of the sediment toxicity tests, the amount of 2,4-dichlorophenol (38 to 23%, respectively, according to Henderson-Hasselbalch equation) were smaller than the ionic 2,4-dichlorophenolate in the water phase. In accordance with pentachlorophenolate, the diffusion of this molecule through biological membranes is hindered, and therefore, the bioavailability is reduced. Due to the lower pH, the amount of nonionic 2,4-DCP was higher in the water phase of the acute toxicity tests (83% to 61 %).

3.7.3 3,4-Dichloroaniline

3.7.3.1 Sediment toxicity test with *L. variegatus*

The sediment toxicity test with *L. variegatus* was done by OETKEN *et al.* (2001). In the highest concentration, no animals survived because of high concentrations measured in pore water and overlying water. Measurements ranged from 10.9 to 19.53 $mg\,l^{-1}$ (OETKEN *et al.*, 2001), which were in the range of LC_{50} (96 h) of 7.1 $mg\,l^{-1}$. NOEC / LOEC values were 0.04 / 0.1 $mg\,kg^{-1}$ (nominal: 5 / 25 $mg\,kg^{-1}$). For the LOEC, the highest measured concentration in pore water was 0.53 $mg\,l^{-1}$, which is 13 fold below the LC_{50} (96 h). Thus, acute toxic effects via the water-solved substance can be disregarded for LOEC at the beginning of the exposure period. Exposure via 3,4-DCA bound to ingested sediment was likely to be the main exposure route.

3.7.3.2 Sediment toxicity test with *C. riparius*

The sediment toxicity test with *C. riparius* was done by OETKEN *et al.* (2001). Effects were observed for the endpoint development rate for all tested concentrations (NOEC / LOEC = - / 0.064 $mg\,kg^{-1}$). Other endpoints showed no effects up to the highest tested concentration. Measurements of overlying water ranged from 0.004 to 0.056 $mg\,l^{-1}$ for all concentrations. Pore water concentrations ranged from below the detection limit of 1.5 $\mu g\,l^{-1}$ to 0.59 $mg\,l^{-1}$ in the highest concentration (OETKEN *et al.*, 2001), which is 10 fold lower than the LC_{50} (48 hour) of 6.1 $mg\,l^{-1}$. Thus, acute toxic effects via the substance in water phase can be disregarded. This result coincides with the fact, that no effects were observed on emergence in the 28-day sediment toxicity test. Effects on development rate very likely occurred via the ingested contaminant.

3.7.3.3 Summary of exposure effect assessment of sediment toxicity tests with 3,4-DCA

Measured concentrations in the LOEC of the sediment toxicity tests were lower by a factor of 10 and 13 than the LC_{50} values of the acute toxicity tests of *C. riparius* and *L. variegatus*, respectively. Thus, the acute toxicity via the water phase at the start of the exposure can be excluded. The main exposure likely took place via the ingestion of sediment and particle-bound 3,4-DCA.

3.7.4 4,4-Dichlorodiphenyltrichloroethan

The analytical measurements of each compartment of selected concentrations are summarized in table 3.15 on page 44.

3.7.4.1 Sediment toxicity test with *L. variegatus*

The lowest NOEC / LOEC values were 2.8 / 34 $mg\,kg^{-1}$ for the total number of worms. The lowest EC_{50} of 48 $mg\,kg^{-1}$ was derived for the endpoint total number of worms. The estimated concentrations of overlying and/ or pore water samples of the lowest 28-day LOEC (concentrations below and above were analyzed) at the start and end of the exposure were in the range of LC_{50} (96 h) of 2.8 $\mu g\,l^{-1}$. Acute toxic effects via DDT solved in water were likely. The estimated concentration in pore water at the start of the exposure was 8.6 $\mu g\,l^{-1}$. In the highest nominal concentration of 500 $mg\,kg^{-1}$, pore water concentrations (28 - 48 $\mu g\,l^{-1}$) were 10 fold higher than LC_{50} (96 h). Thus, observed effects in the highest concentration were very likely due to the acute toxic effects of DDT in the water phase.

3.7.4.2 Sediment toxicity test with *C. riparius*

The lowest NOEC / LOEC values were 0.04 / 0.24 $mg\,kg^{-1}$ for endpoints emergence and total dry weight of the males. The lowest EC_{50} of 0.13 $mg\,kg^{-1}$ was derived for the endpoint total dry weight of male midges. Measured concentrations of overlying and/ or pore water samples of the lowest 28 d LOEC at the start and end of the exposure were below the limit of quantification (lq = 0.1 $\mu g\,l^{-1}$) and lower than the LC_{50} (48 hour) of 0.9 $\mu g\,l^{-1}$. Thus, it can be assumed that acute toxic effects via the water phase can be excluded for the LOEC. The pore water concentration of the highest treatment of 8.1 $mg\,kg^{-1}$ was 0.66 $\mu g\,l^{-1}$ and thus in the range of LC_{50} (48 hour). No emergence was observed in this concentration, which is likely due to the acute toxic effects of DDT in the water phase.

3.7.4.3 Summary of exposure effect assessment of sediment toxicity tests with DDT

According to the analytical measurements, acute toxicity via the water phase may have occurred in the *L. variegatus* sediment toxicity test for the lowest LOEC. For the sediment toxicity test with *C. riparius*, concentrations in the water phase of the lowest LOEC were at least 9 times lower than the observed acute LC_{50} value. Thus, acute toxicity via the water phase can be disregarded for *C. riparius*. Exposure via ingested sediment and particle-bound DDT should have played the main role. *C. riparius* was more sensitive than *L. variegatus*. DDT as an organophosphate insecticide inhibits the natrium channel deactivation (SCHMIDT, 1986), leading to continuous stimulation of the nerves of insects and crustaceans (JCIA, 1997). DDT has primary effects on the nervous system of arthropods. Effects are observed for *C. riparius* even at lower concentrations in the water phases of the sediment toxicity test than effect concentrations of the acute toxicity test. DDT was surprisingly less toxic to *L. variegatus* than to *C. riparius*.

3.7.5 Benzo-[a]-pyrene

The analytical measurements of each compartment of selected concentrations are summarized in table 3.14 on page 43.

3.7.5.1 Sediment toxicity test with *L. variegatus*

The lowest NOEC / LOEC values were 5.9 / 30.2 $mg\,kg^{-1}$. The lowest EC_{50} of 177 $mg\,kg^{-1}$ was derived for the endpoint total dry weight of worms. The highest pore water value of 31 $\mu g\,l^{-1}$ was measured in 28-day NOEC. LOEC was not analyzed. The highest measured value in pore water of the highest tested concentration was 848 $\mu g\,l^{-1}$. No effects were observed

in acute toxicity tests up to 2 $mg\,l^{-1}$. Thus, it can be assumed that acute toxic effects via the water phase can be excluded for all concentrations.

3.7.5.2 Sediment toxicity test with *C. riparius*

The lowest NOEC / LOEC values were 76 / 738 $mg\,kg^{-1}$. The analytical measurements of each compartment of selected concentrations are summarized in table 3.14 on page 43. Measured concentrations of overlying and/ or pore water samples of 28-day LOEC ranged from 0.36 $\mu g\,l^{-1}$ in overlying water at the end to 1600 $\mu g\,l^{-1}$ in pore water at the start of the exposure. No effects were observed in acute toxicity tests up to 2 $mg\,l^{-1}$. Thus, it can be assumed that acute toxic effects via the water phase can be excluded for the LOEC.

3.7.5.3 Summary of exposure effect assessment of sediment toxicity tests with B(a)P

Measured concentrations in the LOEC of the sediment toxicity tests were lower than the highest tested concentration that exhibited no effects in acute toxicity tests for *C. riparius* and *L. variegatus*. Thus, the acute toxicity via the water phase at the start of the exposure can be excluded. The main route of exposure must have been via the ingestion of contaminated sediments. As was shown by (LEPPÄNEN & KUKKONEN, 2006), the dietary uptake of B(a)P by worms was clearly higher than uptake via the water phase only, which can easily be distinguished by using feeding and nonfeeding worms. With the digestion of organic matter in the gut, the dissolved chemical is subsequently increased and thus made bioavailable (LEPPÄNEN & KUKKONEN, 2006).

3.7.6 Tributyltinchloride

The analytical measurements of each compartment of selected concentrations are summarized in table 3.11 on page 38.

3.7.6.1 Sediment toxicity test with *L. variegatus*

NOEC / LOEC values were 0.29 / 1.46 $mg\,kg^{-1}$ TBT-Sn. The lowest EC_{50} of 0.98 $mg\,kg^{-1}$ was derived for the endpoint total dry weight of worms. The estimated concentrations of overlying and/ or pore water samples of the lowest 28-day LOEC (concentrations below and above were analyzed) at the start and end of the exposure exceeded the LC_{50} (96 h) of 4 $\mu g\,l^{-1}$. Estimated concentration in pore water at the start of the exposure was 142 $\mu g\,l^{-1}$. Pore water concentrations as high as 17.5 $\mu g\,l^{-1}$ were measured in the NOEC, which is higher by a factor of 4 than LC_{50} (96 h). Thus, acute toxic effects via the water phase cannot be excluded.

3.7.6.2 Sediment toxicity test with *C. riparius*

The lowest NOEC / LOEC values were 1.46 / 2.92 $mg\,kg^{-1}$ TBT-Sn. The lowest EC_{50} of 1.9 $mg\,kg^{-1}$ was derived for the endpoint total dry weight of female midges. The estimated concentrations of overlying and/ or pore water samples of the lowest 28-day LOEC (concentrations below and above were analyzed) at the start and end of the exposure were below the LC_{50} (48 hour) of 24.6 $\mu g\,l^{-1}$. The estimated overlying/ pore water concentrations ranged from 1.7 to 5.0 $\mu g\,l^{-1}$. Thus, acute toxic effects via the water phase can be excluded. Even at the highest concentration of 16 $mg\,kg^{-1}$ with no emergence, the highest measured value of 11 $\mu g\,l^{-1}$ in pore water was lower by a factor of 2 than LC_{50} (48 hour).

3.7.6.3 Summary of exposure effect assessment of sediment toxicity tests with TBT

Measured concentrations in the LOEC of the sediment toxicity test was 35 times higher for *L. variegatus* than the LC_{50} of the acute toxicity test. For *C. riparius*, pore/ overlying water concentrations were 5 times lower than the LC_{50}. Thus, for *L. variegatus*, it is indicated that toxicity must have mainly occurred via water-solved TBT. For *C. riparius*, results implicate that exposure route via ingested sediment and particle-bound TBT is of higher importance for resulting toxicity. It was obvious that *C. riparius* was the least sensitive organism in the acute toxicity test as well as in the sediment toxicity test. BARTLETT *et al.* (2004a) reported that dissolved TBT is the primary route of exposure for *H. azteca*. However, this study further showed that the uptake rate was significantly higher for sediment-exposed amphipods compared to water-only exposed organisms.

3.7.7 2,4,6-trinitrotoluene

The analytical measurements of each compartment of selected concentrations are summarized in table 3.13 on page 41.

3.7.7.1 Sediment toxicity test with *L. variegatus*

The lowest NOEC / LOEC values were 1.9 / 9.2 $mg\,kg^{-1}$. The lowest EC_{50} of 2.6 $mg\,kg^{-1}$ was derived for the endpoint total number of worms. The concentrations of overlying and/ or pore water samples were below the limit of quantification of 0.004 $mg\,l^{-1}$ in all measured concentrations at the start and end of the exposure period, with the exception of the highest concentration (500 $mg\,kg^{-1}$) at the start of the exposure. An overlying water concentration of 3.55 $mg\,l^{-1}$ was analyzed. Water concentrations were lower than the LC_{50} (96 h) of 9.0 $mg\,l^{-1}$. Thus, acute toxic effects via the water phase can be excluded for the beginning of the exposure period.

3.7.7.2 Sediment toxicity test with *C. riparius*

The lowest NOEC / LOEC values were 0.4 / 1 $mg\,kg^{-1}$ TNT. The lowest EC_{50} of 1.3 $mg\,kg^{-1}$ was derived for the endpoint emergence. The concentrations of overlying and/ or pore water samples were below the limit of quantification of 0.004 $mg\,l^{-1}$ in all measured concentrations at the start and end of the exposure period. Water concentrations were lower than the LC_{50} (48 hour) of 13.7 $mg\,l^{-1}$. Thus, acute toxic effects via the water phase can be excluded.

3.7.7.3 Summary of exposure effect assessment of sediment toxicity tests with TNT

Measured concentrations in the LOEC of the sediment toxicity tests were lower by a factor of 2250 to 3400 than the LC_{50} of the acute toxicity tests for *L. variegatus* and *C. riparius*, respectively. However, it was obvious that TNT disappeared almost completely from water phases (measurements were mainly below the limit of quantification) due to metabolizing, volatilization, and other processes.

It is difficult to study toxicity (and bioaccumulation) of TNT in spiked sediments, because of rapid transformation of the compound (ELOVITZ & WEBER, 1999; CONDER *et al.*, 2004b). Due to the low hydrophobicity, the main exposure route of the uptake of TNT and its metabolites is probably at the dermal interface with water, rather than the intestinal interface with ingested sediment (CONDER *et al.*, 2004a). This assumption was supported in a study with water-only and whole sediment exposure with *Tubifex tubifex*, as toxicity was best described by dissolved nitroaromatic compounds concentrations in pore water (CONDER *et al.*, 2004c). This result is in contrast to the findings of this study, where concentrations in pore and overlying water of effect concentrations were far below the acute toxicity data of water-only tests.

3.7.8 Cadmiumchloride

3.7.8.1 Sediment toxicity test with *L. variegatus*

The lowest NOEC / LOEC values were 4.9 / 24.5 $mg\,kg^{-1}$ Cd. The lowest EC_{50} of 4.4 $mg\,kg^{-1}$ was derived for the endpoint total number of worms. The analytical measurements of each compartment of selected concentrations are summarized in table 3.12 on page 40. No analytical measurements were done for the lowest LOEC. The estimated overlying/ pore water concentrations ranged from 0.26 to 6.3 $mg\,l^{-1}$. The concentrations of overlying and/ or pore water samples were higher up to a factor of 31 than the LC_{50} (48 hour) of 0.2 $mg\,l^{-1}$. Thus, acute toxic effects via the water phase are obvious.

3.7.8.2 Sediment toxicity test with *C. riparius*

The lowest NOEC / LOEC values were 0.012 / 0.12 mg kg^{-1} Cd for the endpoint development rate. The lowest EC_{50} of 7.2 mg kg^{-1} was derived for the endpoint total dry weight of female midges. The analytical measurements of each compartment of selected concentrations are summarized in table 3.12 on page 40. No analytical measurements were done for the lowest LOEC. The estimated overlying/ pore water concentrations ranged from 1 to 155 μg l^{-1}. The maximal concentrations of overlying and/ or pore water samples were below the LC_{50} (48 hour) of 4.8 mg l^{-1}. Thus, acute toxic effects via the water phase can be excluded for the LOEC of the endpoint development rate. The estimated pore water concentrations for the LOEC of the endpoints emergence and dry weight of midges were 15.4 mg l^{-1} for the start and 12.8 mg l^{-1} for the end of the exposure period. These concentrations were higher by a factor of 3 than the LC_{50} (48 hour). Acute toxic effects via solved cadmium in the water phase must be taken into account for this concentration.

3.7.8.3 Summary of exposure effect assessment of sediment toxicity tests with cadmium

The highest measured concentrations in overlying/ pore water of the LOEC of the sediment toxicity test with *L. variegatus* were 31 times higher than the LC_{50} of the acute toxicity test. For the *C. riparius* sediment toxicity test, the development rate was the most sensitive endpoint. Pore water concentration at the LOEC for this endpoint was lower by a factor of 31 than acute LC_{50}. But when comparing the LOEC for endpoint emergence, which is highly relevant for population growth, concentration in pore water was 3 times higher than acute LC_{50}. Thus, exposure via water located-cadmium should mainly cause toxic effects. A relatively high amount of cadmium was found in the water phase according to high water solubility and its properties as an inorganic substance. However, only total Cd concentration was measured in pore and overlying water samples. As outlined in section 3.4 on page 100, only free Cd the ion is bioavailable. Several factors such as organic matter content and AVS can lower the bioavailability. Thus, high concentration in the water phase does not necessarily mean that the total measured Cd was bioavailable.

3.7.9 Summary of exposure effect assessment

In general, the assumptions on which exposure route is causing the effects must be interpreted with caution. Acute toxicity tests via the water phase that lasted for only 48 or 96 hours were used for data comparison. To more realistically assess the exposure route, it would be necessary to compare data of tests with water-only and sediment exposure over the same exposure period. For *L. variegatus*, the selection of feeding (ingesting) and non-feeding (non-ingesting) worms in sediment toxicity tests allows for distinguishing between the exposure via

water-only and natural exposure, including the ingestion of contaminated sediment as used in bioaccumulation studies (LEPPÄNEN & KUKKONEN, 2006). This method would be limited to a 7-day exposure period, the time to regenerate a new head for the non-ingesting worms. Differences in sediment composition of tests with *L. variegatus* and *C. riparius* may influence contaminant concentrations of each compartment and therefore alter exposure. Bioturbation may be different as well for the two test systems. Benthic invertebrates interact directly with sediments. Burrowing activity of tubificid worms released metals (BODDINGTON *et al.*, 1979) and organics (KARICKHOFF & MORRIS, 1985) from sediments. CLEMENTS *et al.* (1994) hypothesized that all the activities of *C. riparius* (such as feeding, tube building, and burrowing) that disturb the upper sediment layer were directly responsible for the remobilization of B(a)P. Higher chironomid densities lead to higher concentrations in overlying water and significantly higher concentrations in larvae, suggesting that resuspended contaminants were bioavailable (CLEMENTS *et al.*, 1994). Differences (bioturbation, sediment composition, contact area between sediment and water, water sediment ratio) in sediment toxicity tests with *L. variegatus* and *C. riparius* have an impact on concentrations in water phases and result in different exposure scenarios.

However, distinguishing the exposure routes by the method used may indicate the true exposure scenario. In general, the tested substances can be divided into 3 classes. Class 1 contains the chemicals for which, in both *L. variegatus* and *C. riparius* sediment toxicity tests, toxicity took place mainly via ingested sediment and particle-bound contaminant. 3,4-DCA, B(a)P, and TNT belong to the substances for which toxic effects are mainly attributed to the ingestion of sediment and particle-bound substances. Class 2 are chemicals for which toxicity took place mainly via water-solved substance for sediment toxicity tests of both invertebrates. 2,4-DCP belongs to this class. Further, cadmium belongs to class 2 when disregarding the effects on the development rate of midges. Class 3 are chemicals for which exposure routes are dependent upon the test organism. For PCP, DDT, and TBT-Cl, the main exposure routes differed for the tested species. In conclusion, effects occur not only by contaminant concentrations in water phases, but also by the ingestion of particle- or sediment-bound contaminants. Exposure routes were species and chemical dependent. Therefore, the prediction of sediment toxicity from acute toxicity data is not possible by water-only concentrations.

3.8 Validation of the new *L. variegatus* sediment for *C. riparius*

Even though sediment compositions were quite similar for *L. variegatus* and *C. riparius*, differences exist, which may have consequences on the organisms' exposure to contaminants. Differences in sediment water partitioning may be attributed to differences (i.e., different sediment to overlying water ratios) of the sediments used. For data comparison, it would be beneficial to use the same sediment composition and the same water-to-sediment ratios for

the two test organisms. Therefore, the improved sediment for *L. variegatus* was tested for *C. riparius* on a bigger scale. This test composition was simultaneously compared to the "older" sediment composition, each replicated 5 times. Results of emergence are shown in table 3.23. Mean total emergence were 89% and 83% for the "old" and the "new" sediment,

Table 3.23: Results of 28-day sediment tests with *C. riparius* in "new" improved sediments for *L. variegatus* and in "old" sediments without test substance

	"old" sediment			"new" sediment		
rep	male	female	emergence [%]	male	female	emergence [%]
1	7	11	90	11	6	85
2	6	11	85	6	8	70
3	9	6	75	9	11	100
4	9	10	95	6	9	75
5	12	8	100	10	7	85
mean	8.6	9.2	89	8.4	8.2	83
median	8.8	9.6	89.5	8.7	8.1	84
stdev	2.3	2.2	9.6	2.3	1.9	11.5
cv (%)	26.8	23.6	10.8	27.4	23.5	13.9
stdev = standard deviation, cv = coefficient of variance						

respectively, and were thus higher than the average emergence of 78% and 82% for controls and solvent controls of all conducted sediment toxicity tests according to the old sediment. Results indicate that there is no difference between the success of emergence of the two sediment compositions. Cumulative emergence patterns (figure 3.53) are in good agreement with each other. These final investigations show that this "new" sediment with low organic carbon and a relatively coarse grain size is suitable for the two benthic invertebrates *L. variegatus* and *C. riparius*. Thus, this sediment with relatively low OC content can be used for sediment toxicity testing of chemicals with sediment accumulation potential.

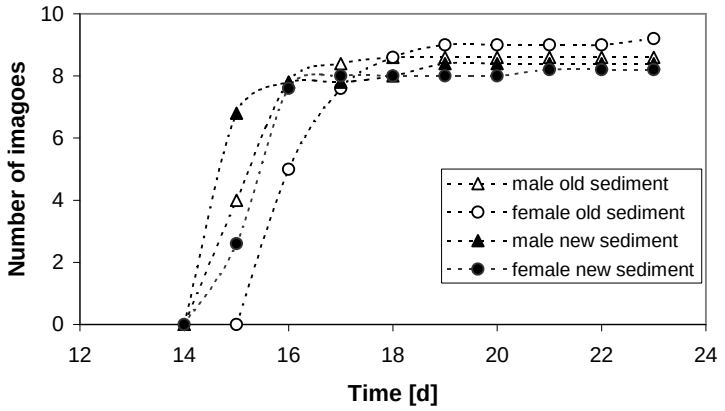


Figure 3.53: Cumulative emergence of *C. riparius* in "new" versus "old" sediment, mean of all replicates

3.9 Validity of sediment toxicity tests

3.9.1 Validation of sediment toxicity test with *L. variegatus*

Prior to testing the selected substances, extensive tests were accomplished to find a suitable sediment composition for good reproduction of *L. variegatus*. The test consistency and reproducibility were evaluated by descriptive statistics for controls and solvent controls, to provide an idea of the variability of the biological endpoints of *L. variegatus* in the test system with the sediment composition used as described in section 2.4.4 on page 17. The statistic parameters for controls and solvent controls of pretests and of toxicity tests are summarized in tables 3.24 and 3.25 for the total number of worms, in tables 3.26 and 3.27 for total biomass and in tables 3.28 and 3.29 for individual dry weight. Water quality parameters are discussed in section 3.9.1.4 on page 129.

3.9.1.1 Worm number

The total number of worms of controls and solvent controls are shown for the individual pretests and sediment toxicity tests in the following table 3.24.

Table 3.24: Results of individual pretests and sediment toxicity tests with *L. variegatus* of controls and solvent controls: Total number of worms, SD = standard deviation, CV = coefficient of variance, n = number

	total worm number of controls [%]						total worm number of solvent controls [%]					
code	mean	min	max	SD	CV(%)	n	mean	min	max	SD	CV(%)	n
1	38.6	23	45	9.5	24.6	5	32.4	22	44	8.0	24.7	5
2	56.2	32	84	20.2	35.9	5	44.0	36	64	11.3	25.8	5
3	48.6	38	61	9.9	20.3	5						
4	34.4	13	51	14.1	41.0	5						
DDT	42.0	25	69	16.8	39.9	5	25.3	11	37	8.9	35.2	6
intertest												
mean	44.0	26.2	62.0		32.4		33.9	23.0	48.3		28.6	
SD	8.6	9.5	15.4		9.4		9.4	12.5	14.0		5.8	
n	5						3					
min	34	13	45		20.3		25.3	11	37		24.7	
max	56.2	38	84		41.0		44	36	64		35.2	
CV(%)	19.5	36.1	24.8		28.9		27.8	54.5	29.0		20.2	

The mean number of worms found in the beakers of controls after 28 days was 44 with a minimum of 13 worms and a maximum of 84 worms. The number of worms inserted (10 worms) at the beginning of the test was increased by 340% based on mean number. The minimum increase observed in one replicate was still 30%. The mean coefficient of variance

Table 3.25: Results of *L. variegatus* sediment toxicity test with PCP of UBA-Ringtest of controls and solvent controls: Total number of worms, SD = standard deviation, CV = coefficient of variance, n = number

	total worm number of controls [%]						total worm number of solvent controls [%]					
code	mean	min	max	SD	CV(%)	n	mean	min	max	SD	CV(%)	n
UBA-Ring	31.2	26	39	5.6	17.9	5	30.7	30	32	1.2	3.8	3

was 32% of all tests with a minimum of 20 % and a maximum of 41%. This means a high variation of the number of worms found within the different replicates.

The mean number of worms found in the beakers of solvent controls after 28 days was 34 with a minimum of 11 worms and a maximum of 64 worms. The number of worms inserted (10 worms) at the beginning of the test was increased by 240% based on mean number. The minimum increase observed in one replicate was 10%. Controls exhibited a better reproduction than solvent controls. But it needs to be taken into account that the solvent controls sample size of three tests with five or more replicates is small. The mean coefficient of variance was 29% of all tests with a minimum of 25% and a maximum of 35%. The variation of solvent controls was also high but lower than the variation of controls.

Statistic parameters for controls and solvent controls of the UBA-ring sediment toxicity test with PCP in which a slightly different sediment composition was used is shown in table 3.25. This test was conducted within the international ring sediment toxicity test in order to develop a OECD guideline funded by the German Federal Environmental Agency. The mean number of worms found in controls and solvent controls was 31.2 and 30.7, respectively, with a minimum of 26 and 30 and a maximum of 39 and 32, respectively. The mean increase after 28-day exposure was 210% for controls and solvent controls, which is less than was found in controls and solvent controls of our sediment. The mean coefficient of variance was 18 % for controls and 4% for solvent controls. This variation was low compared to the variation of controls of our sediment used.

The mean total worm number of all tests was within the required validity criterion for increase in the number of living worms after 28 days by at least 80%. The minimum increase in the number of worms was 130% in solvent controls and 230% in controls of sediment toxicity test with TBT-Cl where only one replicate was used.

3.9.1.2 Worm total biomass

The biomass of worms of controls and solvent controls are shown for the individual pretests and sediment toxicity tests in the following tables 3.26 and 3.27.

The mean dry weight of worms found in the beakers of controls after 28 days was 41 mg with a minimum of 15 mg and a maximum of 70 mg. When assuming an individual mean dry weight of 1 mg per worm inserted at the beginning of the test, the biomass had increased by

Table 3.26: Results of individual pretests and sediment toxicity tests with *L. variegatus* of controls and solvent controls: Total dry weight of worms, SD = standard deviation, CV = coefficient of variance, n = number

	total dry weight of controls [mg]						total dry weight of solvent controls [mg]					
code	mean	min	max	SD	CV(%)	n	mean	min	max	SD	CV(%)	n
1	46.1	32.2	67.2	14.1	30.6	5	20.9	15.1	29.8	5.6	26.9	5
2	43.7	34.2	61.0	10.2	23.4	5	39.0	35.0	46.8	4.8	12.3	5
3	49.0	37.5	69.7	13.7	28.0	5						
4	34.4	15.1	47.0	14.2	41.3	5						
DDT	32.5	22.6	52.8	12.5	38.4	5	17.0	8.1	22.9	5.4	31.8	6
intertest												
mean	41.1	28.3	59.5		32.3		25.6	19.4	33.2		23.7	
SD	7.3	9.2	9.6		7.4		11.7	14.0	12.3		10.1	
n	5						3					
min	33	15.1	47.0		23.4		17.0	8.1	22.9		12.3	
max	49.0	37.5	69.7		41.3		39.0	35.0	46.8		31.8	
CV(%)	17.7	32.7	16.1		22.8		45.8	72.1	37.1		42.9	

Table 3.27: Results of *L. variegatus* sediment toxicity test with PCP of UBA-Ringtest of controls and solvent controls: Total dry weight of worms, SD = standard deviation, CV = coefficient of variance, n = number of replicates

	total biomass of controls [mg]						total biomass of solvent controls [mg]					
code	mean	min	max	SD	CV(%)	n	mean	min	max	SD	CV(%)	n
UBA-Ring	23.2	14.1	28.5	5.4	23.3	5	24.6	20.8	30.4	5.1	20.7	3

310% based on mean. The minimum increase observed in one replicate was still 50 %. The mean coefficient of variance was 32% of all tests with a minimum of 23% and a maximum of 41%. This means a high variation of the total biomass of worms found within the different replicates.

The mean dry weight of worms found in the beakers of solvent controls after 28 days was 26 mg with a minimum of 8 mg and a maximum of 47 mg. When assuming an individual mean dry weight of 1 mg per worm inserted at the beginning of the test, the biomass had increased by 160% based on mean. The minimum increase observed in one replicate was -20%. The mean coefficient of variance was 24% of all tests with a minimum of 12% and a maximum of 31%. The variation of solvent controls was high, also but was lower than the variation of controls.

Statistic parameters for controls of the UBA-ring sediment toxicity test with PCP in which a different sediment composition was used is shown in table 3.27. The mean dry weight of worms found in controls and solvent controls was 23 mg and 25 mg, respectively, with a minimum of 14 mg and 21 mg and a maximum of 29 mg and 30 mg, respectively. The mean increase after 28-day exposure was 130% for controls and 150% for solvent controls, which is less than was found in controls and solvent controls of our sediment with a coarser grain size. The mean coefficient of variance was 23% for controls and 21% for solvent controls. This

variation was smaller compared to the variation of controls and solvent controls of the coarser sediment.

3.9.1.3 Worm individual dry weight

The individual dry weight of worms of controls and solvent controls are shown for the individual pretests and sediment toxicity tests in the following tables 3.28 and 3.29.

Table 3.28: Results of individual pretests and sediment toxicity tests with *L. variegatus* of controls and solvent controls: Individual dry weight of worms, SD = standard deviation, CV = coefficient of variance, n = number

	individual dry weight of controls [mg]						ind. dry weight of solvent controls [mg]					
code	mean	min	max	SD	CV(%)	n	mean	min	max	SD	CV(%)	n
1	1.20	0.95	1.49	0.23	19.4	5	0.64	0.58	0.68	0.04	6.9	5
2	0.82	0.60	1.07	0.18	22.4	5	0.91	0.73	0.98	0.10	11.1	5
3	0.89	0.57	1.08	0.21	23.3	5						
4	1.02	0.81	1.16	0.15	15.1	5						
DDT	0.79	0.57	0.91	0.14	17.8	5	0.68	0.62	0.74	0.05	7.3	6
intertest												
mean	0.94	0.70	1.14		19.6		0.74	0.64	0.80		8.4	
SD	0.17	0.17	0.22		3.3		0.14	0.08	0.16		2.3	
n	5.00						3.00					
min	0.79	0.57	0.91		15.1		0.64	0.58	0.68		6.9	
max	1.20	0.95	1.49		23.3		0.91	0.73	0.98		11.1	
CV(%)	18.0	25.0	18.9		17.0		19.0	12.4	19.6		27.7	

Table 3.29: Results of *L. variegatus* sediment toxicity test with PCP of UBA-Ringtest of controls and solvent controls: Individual dry weight of worms, SD = standard deviation, CV = coefficient of variance, n = number

	individual dry weight of controls [mg]						ind. dry weight of solvent controls [mg]					
code	mean	min	max	SD	CV(%)	n	mean	min	max	SD	CV(%)	n
UBA-Ring	0.75	0.54	0.94	0.15	20.7	5	0.81	0.65	1.01	0.19	23.2	3

The mean individual dry weight of worms found in the beakers of controls after 28 days was 0.94 mg with a minimum of 0.57 mg and a maximum of 1.49 mg. The mean coefficient of variance was 19.6% of all tests with a minimum of 15.1% and a maximum of 23.3%. This means a high variation of individual body dry weight of worms found within the different replicates.

The mean individual dry weight of worms found in the beakers of solvent controls after 28 days was 0.74 mg with a minimum of 0.58 mg and a maximum of 0.98 mg. The mean coefficient of variance was 8.4% of all tests with a minimum of 6.9% and a maximum of 11.1%. The variation of solvent controls was also high, but was lower than the variation of controls.

Statistic parameters for controls of the UBA-ring sediment toxicity test with PCP in which a different sediment composition was used is shown in table 3.29. The mean individual dry weight of worms found in controls and solvent controls was 0.75 and 0.81, respectively, with a minimum of 0.54 and 0.65 and a maximum of 0.94 and 1.01, respectively. The individual dry weight of worms exposed in the OECD control sediment was lower than in controls of our sediment with the coarser grain size. Solvent controls exhibited slightly higher individual dry weight compared to the coarser sediment. The mean coefficient of variance was 20.7% for controls and 23.2% for solvent controls. This variation was higher compared to the variation of controls and solvent controls of tests with the coarser sediment.

3.9.1.4 Water quality parameters

Water quality parameters of overlying water of controls, solvent controls, and treatments are listed in detail in table 7.3 on page 166 in the appendix.

Measured pH values were 8.34 in mean, 7.12 in minimum, and 8.73 in maximum (n = 316). pH values were in the range between 6 and 9, which is required as a validity criterion.

The mean measured oxygen concentration was at 83% of the air saturation value at the start and the end of the exposure period. The minimum measured concentration was 54% of the air saturation value. Only 3 (1%) measurements of 316 were below 60% of the air saturation value. With few exceptions, oxygen concentrations were above 60% of the air saturation value, which is required as a validity criterion.

The mean temperature was 19.98 °C for all tests (316 measurements) with a minimum of 17.9 °C and a maximum of 21 °C. Of the measured temperatures, 7% were below 19 °C.

The mean conductivity was 754 $\mu S\,cm^{-1}$ for all tests (316 measurements) with a minimum of 545 $\mu S\,cm^{-1}$ and a maximum of 1164 $\mu S\,cm^{-1}$.

3.9.2 Validation of sediment toxicity test with *C. riparius*

The test consistency and reproducibility were evaluated by descriptive statistics for controls and solvent controls. This was done to provide an idea of the variability of the biological endpoints of *C. riparius* in the test system with the sediment composition used as described in section 2.4.2 on page 14. The statistic parameters for controls and solvent controls of all sediment toxicity tests are summarized in tables 3.30 for total emergence, 3.31 for biomass of emerged midges, 3.32 for individual dry weight, and 3.33 for development rate. Water quality parameters are discussed in section 3.9.2.5 on page 136.

3.9.2.1 Emergence of *C. riparius*

The total emergence of *C. riparius* of controls and solvent controls are shown for the individual sediment toxicity tests in the following table 3.33.

Table 3.30: Results of individual test runs of sediment toxicity test with *C. riparius* of controls and solvent controls: Total emergence, SD = standard deviation, CV = coefficient of variance, n = number

	total emergence of controls [%]						total emergence of solventcontrols [%]					
subst.	mean	min	max	SD	CV(%)	n	mean	min	max	SD	CV(%)	n
2,4-DCP	72	0	100	41.6	57.8	5	44	5	80	30.7	69.8	5
B(a)p	88	75	100	11.5	13.1	5	83	65	95	14.1	17.0	6
Cd	90	70	100	11.7	13.0	5	97	95	100	2.7	2.8	5
Cd*	67	35	95	26.6	39.7	5	95	90	100	3.5	3.7	5
DDT	38	0	80	31.3	82.5	5	96	85	100	6.5	6.8	5
PCP	85	45	100	24.0	28.2	5	92	80	100	8.4	9.1	5
TBT-Cl	91	65	100	15.2	16.7	5	59	30	95	33.1	56.0	5
TNT	91	60	100	18.2	20.0	5	90	75	100	9.4	10.4	5
intertest												
mean	77.8	43.8	96.9		33.9		81.9	65.6	96.3		22.0	
SD	18.5	30.0	7.0		25.0		19.7	31.8	6.9		25.9	
n	8						8					
min	38	0	80		13.0		44	5	80		2.8	
max	91	75	100		82.5		97	95	100		69.8	
CV(%)	23.8	68.5	7.3		73.7		24.1	48.4	7.2		118.0	

* = second test

Controls

The mean emergence of *C. riparius* in the beakers of controls was 78% with a minimum of 0% in one replicate of the toxicity test with 2,4-DCP (cannot be explained) and in one replicate of the test with DDT (due to failure in aeration leading to anoxic conditions in water). A maximum of 100% was observed in 75% of the sediment toxicity tests. The mean coefficient of variance was 34% of all tests with a minimum of 13% and a maximum of 83%. These values demonstrate a high variation of emergence within the different replicates.

Solvent controls

The mean emergence of *C. riparius* in the beakers of solvent controls was 82% with a minimum of 5% in one replicate of the toxicity test with 2,4-DCP. A maximum of 100% was observed in 62.5% of the sediment toxicity tests. The total emergence of *C. riparius* in solvent controls was higher than in controls. Midges emerged in all replicates of solvent controls. There were no replicates without any emergence. The mean coefficient of variance was 22% of all tests with a minimum of 3% and a maximum of 70 %. The variation of solvent controls was high also, but was lower than the variation of controls.

The validity criterion of a mean total emergence of at least 50% in solvent controls was fulfilled for all tests, with one exception. In the sediment toxicity test with 2,4-DCP, the mean total emergence was 44% with a median value of 55%, due to a very small emergence of 5 % in one replicate. This can be explained by a failure in aeration due to technical reasons. Failure in aeration led to a deficit of oxygen, which may had led to mortality of the larvae. Therefore, the test was considered valid. Controls of this test showed a mean emergence of 72% with a median of 85%.

3.9.2.2 Biomass of *C. riparius*

The mean dry weight for male and female *C. riparius* of controls and solvent controls are shown for the individual sediment toxicity tests in table 3.31. The number of emerged midges is reflected in biomass. Reasons for low or little biomass were described in the previous section.

Controls

The mean total biomass of male *C. riparius* in the beakers of controls was 5.3 mg with a minimum of 0 mg in one replicate of the toxicity test with 2,4-DCP and in one replicate of the test with DDT. A maximum of 9 mg was observed in the sediment toxicity test with PCP. The mean coefficient of variance was 46% of all tests with a minimum of 16% and a maximum of 76%. These values demonstrate a high variation of biomass of male *C. riparius* within the different replicates.

The mean total biomass of female *C. riparius* in the beakers of controls was 11.1 mg with a minimum of 0 mg in one replicate of the toxicity test with 2,4-DCP and in one replicate of the test with DDT. A maximum of 21.5 mg was observed in controls of the sediment toxicity test with cadmiumchloride (test 1). The mean coefficient of variance was 47% of all tests with a minimum of 16 % and a maximum of 133% in the sediment toxicity test with DDT. These values demonstrate a high variation of biomass of female *C. riparius* within the different replicates.

Solvent controls

The mean total biomass of male *C. riparius* in the beakers of solvent controls was 5.6 mg with a minimum of 0 mg in one replicate of the toxicity test with 2,4-DCP. A maximum of 11.3 mg was observed in the sediment toxicity test with TBT-Cl. The mean total biomass of male *C. riparius* in solvent controls was slightly higher than in controls. The mean coefficient of variance was 37% of all tests with a minimum of 15% and a maximum of 77%. These values demonstrate a high variation of biomass of male *C. riparius* within the different replicates of solvent controls.

The mean total biomass of female *C. riparius* in the beakers of solvent controls was 12.2 mg with a minimum of 0 mg in one replicate of the toxicity test with 2,4-DCP. A maximum of

Table 3.31: Results of individual test runs of sediment toxicity test with *C. riparius* of controls and solvent controls: Mean dry weight, SD = standard deviation, CV = coefficient of variance, n = number, m = male, f = female

		dry weight of controls [mg]						dry weight of solvent controls [mg]					
sex	subst.	mean	min	max	SD	CV(%)	n	mean	min	max	SD	CV(%)	n
male	2,4-DCP	5.2	0.0	7.8	3.1	59.4	5	1.7	0.0	3.6	1.3	77.4	5
	B(a)p	6.7	3.9	7.8	1.6	23.3	5	5.9	4.0	7.6	1.4	23.5	6
	CdCl2	4.7	1.1	8.2	2.7	57.1	5	5.6	3.7	7.4	1.5	26.6	5
	CdCl2*	4.6	1.8	8.2	2.6	57.1	5	7.2	4.5	10.3	2.3	32.4	5
	DDT	2.5	0.0	4.4	1.9	76.2	5	6.6	3.5	8.5	2.0	30.9	5
	PCP	5.2	2.9	9.0	2.7	51.6	5	7.0	6.2	8.3	1.1	15.1	5
	TBT-Cl	7.1	5.2	7.9	1.1	15.9	5	5.6	4.0	11.3	3.2	57.2	5
	TNT	6.3	3.7	7.5	1.5	24.4	5	4.8	3.1	7.4	1.7	35.5	5
	intertest												
	mean	5.3	2.3	7.6		45.6		5.6	3.6	8.0		37.3	
	SD	1.46	1.93	1.36		21.6		1.75	1.72	2.29		20.2	
	n	8						8					
	min	2.5	0.0	4.4		15.9		1.7	0.0	3.6		15.1	
	max	7.1	5.2	9.0		76.2		7.2	6.2	11.3		77.4	
	CV(%)	27.6	82.9	17.9		47.3		31.4	47.8	28.5		54.2	
female	2,4-DCP	10.3	0.0	15.4	6.1	58.5	5	9.9	0.0	18.6	7.5	75.5	5
	B(a)p	11.8	7.9	13.9	2.4	20.5	5	12.0	8.9	16.2	3.2	26.6	6
	CdCl2	13.3	6.7	21.5	6.0	45.0	5	14.6	11.9	17.5	2.4	16.6	5
	CdCl2*	10.0	5.5	17.5	4.8	47.3	5	13.7	8.3	20.3	4.6	33.3	5
	DDT	5.0	0.0	15.5	6.6	133.3	5	13.1	8.9	18.7	4.3	32.4	5
	PCP	12.2	7.0	18.1	4.0	33.2	5	10.7	8.2	14.8	2.6	24.7	5
	TBT-Cl	12.9	10.1	15.1	2.0	15.8	5	7.6	1.5	19.6	7.7	101.1	5
	TNT	13.5	10.3	17.1	2.6	19.0	5	15.8	5.9	19.6	5.7	36.0	5
	intertest												
	mean	11.1	5.9	16.7		46.6		12.2	6.7	18.2		43.3	
	SD	2.81	4.02	2.36		38.2		2.7	4.03	1.88		29.2	
	n	8						8					
	min	5.0	0.0	13.9		15.8		7.6	0.0	14.8		16.6	
	max	13.5	10.3	21.5		133.3		15.8	11.9	20.3		101.1	
	CV(%)	25.2	67.6	14.1		82.1		22.2	60.2	10.4		67.5	

* = second test

20.3 mg was observed in the sediment toxicity test with cadmiumchloride (test 2). The mean total biomass of female *C. riparius* in solvent controls was slightly higher than in controls. The mean coefficient of variance was 43 % of all tests with a minimum of 17% and a maximum of 101%. These values demonstrate a high variation of biomass of female *C. riparius* within the different replicates of solvent controls.

3.9.2.3 Individual dry weight of *C. riparius*

The mean individual dry weight for male and female *C. riparius* of controls and solvent controls are shown for the individual sediment toxicity tests in the following table 3.32.

Controls

The mean individual dry weight of male *C. riparius* in the beakers of controls was 0.67 mg with a minimum of 0.35 mg and a maximum of 0.89 mg. The mean coefficient of variance was 12% of all tests with a minimum of 4% and a maximum of 21%. These values demonstrate a low variation of individual dry weight of male *C. riparius* within the different replicates.

The mean individual dry weight of female *C. riparius* in the beakers of controls was 1.43 mg with a minimum of 0.75 mg and a maximum of 1.83 mg. The mean coefficient of variance was 9% of all tests with a minimum of 3% and a maximum of 22%. These values demonstrate a low variation of individual dry weight of female *C. riparius* within the different replicates of controls.

Solvent controls

The mean individual dry weight of male *C. riparius* in the beakers of solvent controls was 0.69 mg with a minimum of 0.53 mg and a maximum of 1.25 mg. The mean individual dry weight of male *C. riparius* in solvent controls coincided with the values found for controls. The mean coefficient of variance was 9% of all tests with a minimum of 3% and a maximum of 30%. These values demonstrate a low variation of individual dry weight of male *C. riparius* within the different replicates of solvent controls.

The mean individual dry weight of female *C. riparius* in the beakers of solvent controls was 1.48 mg with a minimum of 1.17 mg and a maximum of 1.75 mg. The mean individual dry weight of female *C. riparius* in solvent controls coincided with the values found for controls. The mean coefficient of variance was 7% of all tests with a minimum of 3% and a maximum of 19%. These values demonstrate a low variation of individual dry weight of female *C. riparius* within the different replicates of solvent controls.

Table 3.32: Results of individual test runs of sediment toxicity test with *C. riparius* of controls and solvent controls: Individual dry weight, SD = standard deviation, CV = coefficient of variance, n = number, m = male, f = female

		individual dry weight of controls [mg]						ind. dry weight of solvent controls [mg]					
sex	subst.	mean	min	max	SD	CV(%)	n	mean	min	max	SD	CV(%)	n
m	2,4-DCP	0.69	0.62	0.75	0.05	7.8	4	0.87	0.70	1.25	0.26	29.5	4
	B(a)p	0.71	0.65	0.80	0.06	8.7	5	0.66	0.64	0.69	0.02	2.7	6
	CdCl2	0.54	0.35	0.63	0.11	20.5	5	0.62	0.53	0.67	0.06	9.1	5
	CdCl2*	0.77	0.72	0.89	0.07	8.7	5	0.72	0.70	0.75	0.02	2.8	5
	DDT	0.55	0.40	0.63	0.11	19.4	4	0.64	0.58	0.71	0.05	7.3	5
	PCP	0.71	0.64	0.82	0.07	10.5	5	0.65	0.62	0.69	0.03	4.8	5
	TBT-Cl	0.75	0.63	0.88	0.11	15.1	5	0.72	0.70	0.79	0.04	5.6	5
	TNT	0.68	0.66	0.73	0.03	4.3	5	0.67	0.62	0.73	0.05	6.9	5
	intertest												
	mean	0.67	0.58	0.77		11.9		0.69	0.63	0.79		8.6	
	SD	0.09	0.13	0.10		5.84		0.08	0.06	0.19		8.73	
	n	8						8					
	min	0.54	0.35	0.63		4.3		0.62	0.53	0.67		2.7	
	max	0.77	0.72	0.89		20.5		0.87	0.70	1.25		29.5	
	CV(%)	12.7	22.7	13		49.2		11.7	9.68	24.4		102	
f	2,4-DCP	1.47	1.44	1.54	0.04	2.94	4	1.49	1.18	1.75	0.27	18.5	4
	B(a)p	1.48	1.35	1.57	0.09	6.13	5	1.50	1.42	1.60	0.06	4.1	6
	CdCl2	1.21	0.75	1.36	0.26	21.58	5	1.40	1.32	1.49	0.07	5.0	5
	CdCl2*	1.65	1.57	1.83	0.10	6.36	5	1.57	1.52	1.66	0.05	3.4	5
	DDT	1.25	1.01	1.55	0.28	21.99	3	1.46	1.39	1.49	0.04	3.0	5
	PCP	1.42	1.34	1.51	0.07	4.79	5	1.40	1.17	1.50	0.14	9.7	5
	TBT-Cl	1.47	1.37	1.58	0.08	5.30	5	1.54	1.51	1.63	0.05	3.3	5
	TNT	1.50	1.45	1.55	0.04	2.80	5	1.47	1.39	1.57	0.07	5.0	5
	intertest												
	mean	1.43	1.29	1.56		9.0		1.48	1.36	1.59		6.5	
	SD	0.14	0.27	0.13		8.01		0.06	0.13	0.09		5.3	
	n	8						8					
	min	1.21	0.75	1.36		2.8		1.40	1.17	1.49		3.0	
	max	1.65	1.57	1.83		22.0		1.57	1.52	1.75		18.5	
	CV(%)	9.76	21.2	8.25		89.1		4.06	9.84	5.77		81.6	

* = second test

3.9.2.4 Development rate of *C. riparius*

The mean development rates for male and female *C. riparius* of controls and solvent controls are shown for the individual sediment toxicity tests in table 3.33.

Table 3.33: Results of individual test runs of sediment toxicity test with *C. riparius* of controls and solvent controls: Development rate, SD = standard deviation, CV = coefficient of variance, n = number, m = male, f = female

		development rate of controls						development rate of solventcontrols					
sex	subst.	mean	min	max	SD	CV(%)	n	mean	min	max	SD	CV(%)	n
m	2,4-DCP	0.061	0.051	0.068	0.0072	11.9	4	0.049	0.046	0.053	0.0035	7.1	4
	B(a)p	0.066	0.062	0.071	0.0040	6.0	5	0.069	0.066	0.072	0.0022	3.2	6
	CdCl2	0.063	0.059	0.066	0.0024	3.9	5	0.062	0.059	0.064	0.0022	3.6	5
	DDT	0.054	0.052	0.058	0.0027	5.0	4	0.055	0.050	0.061	0.0044	8.0	5
	PCP	0.064	0.055	0.071	0.0075	11.8	5	0.062	0.054	0.070	0.0071	11.4	5
	TBT-Cl	0.063	0.057	0.067	0.0038	6.1	5	0.056	0.052	0.064	0.0050	8.9	5
	TNT	0.062	0.056	0.065	0.0035	5.7	5	0.060	0.055	0.065	0.0044	7.2	5
	intertest												
	mean	0.062	0.056	0.066		7.2		0.059	0.055	0.064		7.0	
	SD	0.004	0.004	0.004		3.2		0.006	0.006	0.006		2.9	
	n	7						7					
	min	0.054	0.051	0.058		3.9		0.049	0.046	0.053		3.2	
	max	0.066	0.062	0.071		11.9		0.069	0.066	0.072		11.4	
	CV(%)	6.3	7.2	6.6		45.3		10.7	11.8	9.4		40.7	
f	2,4-DCP	0.056	0.050	0.061	0.0046	8.2	4	0.052	0.050	0.054	0.0018	3.4	4
	B(a)p	0.061	0.058	0.065	0.0030	4.8	5	0.063	0.061	0.066	0.0024	3.8	6
	CdCl2	0.060	0.056	0.064	0.0030	4.9	5	0.058	0.056	0.059	0.0011	2.0	5
	DDT	0.053	0.052	0.054	0.0009	1.7	3	0.051	0.045	0.056	0.0045	8.9	5
	PCP	0.058	0.050	0.064	0.0071	12.2	5	0.056	0.050	0.064	0.0067	11.8	5
	TBT-Cl	0.058	0.050	0.064	0.0053	9.1	5	0.053	0.050	0.058	0.0036	6.8	5
	TNT	0.056	0.049	0.060	0.0041	7.2	5	0.056	0.051	0.061	0.0036	6.4	5
	intertest												
	mean	0.058	0.052	0.062		6.9		0.056	0.052	0.060		6.2	
	SD	0.003	0.003	0.004		3.4		0.004	0.005	0.004		3.4	
	n	7						7					
	min	0.053	0.049	0.054		1.7		0.051	0.045	0.054		2.0	
	max	0.061	0.058	0.065		12.2		0.063	0.061	0.066		11.8	
	CV(%)	4.8	6.6	6.2		49.4		7.4	9.7	7.1		55.7	

Controls

The mean development rate of male *C. riparius* in the beakers of controls was 0.062 with a minimum of 0.051 and a maximum of 0.071. The mean coefficient of variance was 7% of all tests with a minimum of 4 % and a maximum of 12%. These values demonstrate a low variation of development rate of male *C. riparius* within the different replicates.

The mean development rate of female *C. riparius* in the beakers of controls was 0.058 with a minimum of 0.049 and a maximum of 0.065. The mean coefficient of variance was 7% of all tests with a minimum of 2% and a maximum of 12%. These values demonstrate a low variation of development rate of female *C. riparius* within the different replicates of controls.

Solvent controls

The mean development rate of male *C. riparius* in the beakers of solvent controls was 0.059 with a minimum of 0.046 and a maximum of 0.072. The mean development rate of male *C. riparius* in solvent controls coincided with the values found for controls. The mean coefficient of variance was 7% of all tests with a minimum of 3% and a maximum of 11%. These values demonstrate a low variation of development rate of male *C. riparius* within the different replicates of solvent controls.

The mean development rate of female *C. riparius* in the beakers of solvent controls was 0.056 with a minimum of 0.045 and a maximum of 0.066. The mean development rate of female *C. riparius* in solvent controls coincided with the values found for controls. The mean coefficient of variance was 6% of all tests with a minimum of 2% and a maximum of 12%. These values demonstrate a low variation of development rate of female *C. riparius* within the different replicates of solvent controls.

3.9.2.5 Water quality parameters

Water quality parameters of overlying water of controls, solvent controls, and treatments are listed in detail in table 7.4 on page 169 in the appendix.

Measured pH values were 8.42 in mean, 7.04 in minimum, and 8.90 in maximum (n = 523). pH values were in the range between 6 and 9, which is required as a validity criterion.

The mean measured oxygen concentration was at 79% of the air saturation value at the start and the end of the exposure period. The minimum measured concentration was 30% of the air saturation value. Only 19 (3.6%) measurements of 533 were below 60% of the air saturation value. With few exceptions, oxygen concentrations were above 60% of the air saturation value, which is required as a validity criterion.

The mean temperature was 19.85 °C for all tests (533 measurements) with a minimum of 18.4 °C and a maximum of 21.2 °C. Of the measured temperatures, 8.6% were below 19 °C, and 0.2% (1 measurement) were above 21 °C.

The mean conductivity was 970 $\mu S\,cm^{-1}$ for all tests (526 measurements) with a minimum of 764 $\mu S\,cm^{-1}$ and a maximum of 1191 $\mu S\,cm^{-1}$.

3.10 Outlook for sediment toxicity test methods

Sediment composition

Sediment composition for the three tested invertebrates was slightly different according to the detailed description in section 2.4. The overall problem for the sediment tests with invertebrates was the development of a so called "Kahmhaut". Kahmhaut is a biofilm layer composed of bacteria and actinomycetes, and the sheathed bacteria *Sphaerotilus natans*, algae, and protozoa that develops on the interface between overlying water and sediment. These biofilms may interrupt the exchange of gases between sediment and overlying water, which may result in anaerobic zones containing toxic substances such as hydrosulphide and methane. Such anoxic zones lead to unfavorable conditions for the invertebrates. What can be done to prevent Kahmhaut biofilm development? Sufficient aeration, specially during the sediment aging period, is a crucial factor to meet the strong oxygen demand. Also, reduction of easily biodegradable organic matter within the sediment prevents fast-growing biofilms (Kahmhaut). Less oxygen demand and thus favorable test conditions may be achieved. Finally, higher water-to-sediment ratio minimizes the influence of food source onto overlying water quality (BORGMANN & NORWOOD, 1999).

Outlook for *L. variegatus* sediment toxicity test

The sediment toxicity test with *L. variegatus*, according to the used method, exhibited very good reproduction after 28 days. Mean reproduction of 340% in controls and 240% in solvent controls were achieved. There was a high variation within the replicates of controls and solvent controls as well as in treatments. In order to reduce this variation, steps are necessary to stabilize the test system. Better results concerning the coefficient of variance were achieved within the UBA-ring sediment toxicity test (EGELER *et al.*, 2005) using different sediment composition. Within the UBA-ring sediment toxicity test, a lower reproduction compared to the sediment with the coarser grain size was observed. Within the fine particle sediment, according to the new proposed OECD draft guideline (OECD, 2006), kaolin and sphagnum peat were additionally used compared to the coarser sediment. The amount of fresh organic matter/ leaf material was higher in the coarser sediment. This may have lead to higher oxygen demand at the beginning of the sediment aging period. The sometimes observed Kahmhaut may have adverse effects on successful reproduction as described above. The recommended sediment composition for the proposed OECD draft guideline (OECD, 2006) with relatively low variation is suitable when higher amounts of fine particles and organic carbon are required, even though lower reproduction (mean of controls and solvent controls = 210%) was observed for this sediment. Further investigations would be necessary to find a sediment composition containing a food source with a small amount of easily degradable organic contents but still of high quality to serve as food to ensure positive reproduction.

Outlook for *C. riparius* sediment toxicity test

Good survival/ emergence were observed for most tests. Nevertheless, there was a high variation within the replicates of controls, solvent controls, and treatments. The relatively high amount of fresh leaf material contains easily degradable organic matter, which is metabolized by fast growing microorganisms, leading to high oxygen demand during the first test period. Toxic anorganic substances such as nitrate, ammonia, and nitrite are leached into the pore and overlying water. Oxygen demand is very high especially in pore water during sediment aging time. Anoxic zones lead to unfavorable conditions for the larvae. The sometimes observed Kahmhaut may have adverse effects on successful reproduction as described above. In order to reduce these unfavorable conditions and variation in emergence, the organic matter composition should be improved in future investigations. The objective is to use organic matter with a small amount of easily degradable organic substances, preventing fast growing microorganisms and/ or biofilms and resulting in little oxygen demand at the beginning of the test. This improved organic matter must still meet the criteria for a good food source. One recommendation could be the usage of leached organic material. The leaching process is described in detail in the work of LEPPCHEN (2002). This process leaches easily degradable organic and inorganic matter. Thus, oxygen demand can be reduced in pore and overlying water during the first test period.

To improve overlying water quality, higher water sediment ratios may be used, as reported for sediment tests with *C. riparius*, *Heptagenia* spec., *H. azteca*, and *Tubifex tubifex* (water sediment ratios of 64 to 1) (BORGMANN & NORWOOD, 1999). Higher sediment water ratio minimizes the influence of the food source (carbon source) on the overlying water quality. Tests with higher water-to-sediment ratio than 4 to 1 may improve overlying water quality. Thus, one possibility would be to use the sediment composition that was used in the *L. variegatus* sediment toxicity test with higher water-to-sediment ratio and leaves of *U. dioica* and α-cellulose, and excluding the leaves of *A. glutinosa*. This was done in final investigations. This "new" sediment yielded high emergence rates above 80% and may thus be suitable for further sediment toxicity testing.

4 Summary

Seven organic chemicals (TNT, 3,4-DCA, 2,4-DCP, PCP, B(a)P, DDT, and TBT) and one metal compound (cadmium) were selected as model compounds.

Acute toxicity tests were performed with the benthic invertebrates *L. variegatus* and *C. riparius* for the eight selected chemicals. Calculated LC_{50} values covered a range of 4 orders of magnitude. The highest acute toxicity was observed for DDT on *C. riparius* with an LC_{50} (48 h) of 0.9 $\mu g l^{-1}$ DDT, whereas the lowest acute toxicity was observed for 2,4-DCP on *L. variegatus* with a LC_{50} (96 h) of 9.9 $mg l^{-1}$ 2,4-DCP.

Comparison of LC_{50} values for *C. riparius*, *L. variegatus* and literature data of *D. magna* for the tested chemicals indicates that no species was consistently the most sensitive to the eight chemicals. Based on effective concentrations, *D. magna* was the most sensitive species for TBT-Sn, cadmium, and 3,4-DCA. *C. riparius* was the most sensitive species for DDT. *L. variegatus* was the most sensitive species for PCP. 2,4-DCP was equally toxic to *C. riparius* and *D. magna*. 3,4-DCA was equally toxic to *C. riparius* and *L. variegatus*. DDT and TBT turned out to be the most toxic of the tested substances in acute toxicity tests.

Prediction of acute toxicity for the two invertebrates from *D. magna* acute toxicity data was investigated. Data of *D. magna* significantly ($p \leq 0.05$) correlate with effective data of the two tested invertebrates. A prediction of toxicity for *L. variegatus* and *C. riparius* based on *D. magna* data is questionable, due to the small data set and the high variation in sensitivity of the test organisms.

Twenty-eight-day sediment toxicity tests were performed with the benthic invertebrates *L. variegatus* and *C. riparius* for the eight selected chemicals. For *C. riparius* and *L. variegatus*, two different low carbon containing artificial sediments varying in its organic matter composition and water-to-sediment ratios were used for testing. Test systems contained complete food sources so that no additional feeding during the sediment toxicity test was necessary; thus, natural exposure conditions were represented. Further, for *L. variegatus*, PCP was additionally tested with an artificial sediment according to a new proposed OECD draft guideline. However, the sediments that were used for the two organisms to test the selected model substances differed in sediment composition. Therefore, a sediment with the same sediment composition and the same water-to-sediment ratio for both invertebrates was developed, to have similar exposure conditions. Investigations showed that one sediment with low organic carbon and a relatively coarse grain size is suitable for the two benthic invertebrates *L. variegatus* and *C. ri-*

parius and can be used for sediment toxicity testing of chemicals with sediment accumulation potential.

For the selected substances, the lowest effect concentrations were observed for 3,4-DCA with LOEC values of 0.003 $mg\,kg^{-1}$ and 0.1 $mg\,kg^{-1}$ for *C. riparius* and *L. variegatus* (OETKEN *et al.*, 2001), whereas effect concentrations were the highest for B(a)P with LOEC values of 738 $mg\,kg^{-1}$ and 30.4 $mg\,kg^{-1}$ for *C. riparius* and *L. variegatus*. Comparison of effect data of sediment toxicity tests for *C. riparius* and *L. variegatus* for the tested chemicals indicates that no species was consistently more sensitive to the eight chemicals. *C. riparius* was the more sensitive species for four of the eight tested chemicals. *L. variegatus* was more sensitive for three of eight tested chemicals, but differences were smaller than a factor of 5. The toxicity of PCP is nearly the same for the two invertebrates. If differences smaller than a factor of 5 are disregarded, then *C. riparius* is more sensitive than *L. variegatus*.

When comparing sediment toxicity data of *L. variegatus* with *C. riparius*, no significant correlation was observed. This leads to the conclusion that sediment toxicity data may not be extrapolated from one to the other sediment species by this method.

Acute toxicity data was correlated with sediment toxicity data for each organism and possible extrapolation was discussed. Acute 96-hour LC_{50} data of *L. variegatus* significantly correlate with 28-day EC_{50} data of *L. variegatus* sediment toxicity test ($p \leq 0.05$) based on nominal concentrations, whereas no significant correlation was observed for data based on effective concentrations. Thus, a prediction of sediment toxicity is not meaningful. Prediction of sediment toxicity data for *C. riparius* from acute toxicity data would not be possible because of the absence of significant correlation between acute and sediment toxicity data. In conclusion, it is not possible with the method used to predict sediment toxicity from acute toxicity data because there is no correlation between acute and sediment toxicity data.

Partition coefficients for sediment pore and overlying water partitioning were calculated from analytical measurements. Overall, the highest partition coefficients for sediment water partitioning were observed for B(a)P (356,000 l/kg), DDT (65,000 l/kg), and TBT (2500 l/kg), which are the chemicals with high log K_{ow}. Whereas, the lowest were observed for chemicals with relatively lower log K_{ow} and higher water solubility. In general, contaminant pore water concentrations were higher than overlying water concentrations. Observed differences between partition coefficients for *L. variegatus* and *C. riparius* sediment toxicity tests depend on differences in sediment composition (different OC contents) and biological factors. The activities of the animals may affect bioturbation, and thus the exchange processes between compartments.

To get an indication of which exposure route (ingested sediment, pore water, or overlying water) drives chronic toxicity in sediment toxicity tests, substance concentrations in sediment, pore water, and overlying water were analytically determined. Possible exposure routes were examined for all tested chemicals. Therefore, chemicals' acute toxicity via the water phase was compared with concentrations of pore and overlying water of the LOEC of the chronic

28-day sediment toxicity test. If analysis of pore and overlying water of the LOEC of the 28-day sediment toxicity test showed concentrations higher or equal to the calculated LC_{50} of the acute toxicity test (exposure via the water phase), the exposure via the water phase may consequently be the main reason for the observed effects. The tested substances can be divided into three classes. Class 1 are chemicals for which in both *L. variegatus* and *C. riparius* sediment toxicity tests, the toxicity took place mainly via ingested sediment and particle-bound contaminant. 3,4-DCA, B(a)P, and TNT belong to the substances for which toxic effects are mainly attributed to the ingestion of sediment and particle-bound substance. Class 2 are chemicals for which toxicity was mainly due to water solved substances for sediment toxicity tests of both invertebrates. 2,4-DCP belongs to this class. Further, cadmium belongs to class 2 when disregarding the effects on the development rate of midges. Class 3 are chemicals for which exposure routes are dependent upon the test organism. Exposure routes are species-dependent. For PCP, DDT, and TBT-Cl, the main exposure routes differed for the tested species. Results of the exposure effect assessment showed clearly that for the assessment of the results of sediment toxicity tests, measured concentrations of the test compound for each compartment are necessary. Based on the measurements of all compartments, the exposure of the test organisms to the contaminants and their possible degradation products can be accurately described.

General summary and conclusion

Acute toxicity data of the eight tested chemicals of *D. magna* significantly correlate with data of *L. variegatus* and *C. riparius* ($p < 0.05$). However, a prediction of toxicity based on *D. magna* data bears high uncertainty, due to the small data set and high variation in sensitivity of the organisms. Existing sediment toxicity test methods were improved to meet the demand for artificial sediments containing organic matter that serves sufficiently as internal food source for the test organisms, and thus representing natural exposure conditions. However, the sediments that were used for the two organisms to test the selected model substances differed in sediment composition. Therefore, a sediment with the same sediment composition and the same water-to-sediment ratio for both invertebrates was developed, to have similar exposure conditions. In sediment toxicity tests, *C. riparius* was observed to be more sensitive than *L. variegatus*, and no correlation was observed between the data of the invertebrates. For the selected substances, lowest effect concentrations were observed for 3,4-dichloroaniline, whereas effect concentrations were the highest for benzo[a]pyrene. No correlations were found between the acute toxicity data of exposure via the water phase and sediment toxicity data, thus making a prediction of sediment toxicity data impossible. From analytical measurements of chemicals concentration in the compartments overlying, pore water, and bulk sediment, partition coefficients on sediment water partitioning were calculated. The highest partition coefficient ratios for sediment water partitioning were found for the high lipophilic organic substances 4,4-dichlorodiphenyltrichloroethan (DDT) and benzo[a]pyrene. Further, it was found that the main exposure routes in the 28-day sediment toxicity tests were not only chemical but species-dependent. As a result of very differing exposure routes for the tested chemicals and the absence of correlations between the acute and sediment toxicity data, sediment toxicity tests are necessary to assess the toxicity of chemicals on sediment inhabiting organisms.

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7 Appendix

Table 7.1: Physical and chemical variables of acute toxicity tests with *L. variegatus* at beginning (t0h) and end of the exposure(t96h)

chemical	date	time [h]	concentration [mgl^{-1}]	pH	oxygen [mgl^{-1}]	temperature [°C]
2,4-DCP	21-Jul-03	0	c	7.62	10.71	
2,4-DCP	21-Jul-03	0	8.84	7.27	10.24	
2,4-DCP	21-Jul-03	0	50	6.98	9.74	
$CdCl_2$	08-Jul-02	0	c	7.4	8.7	
$CdCl_2$	08-Jul-02	0	0.206	7.49	8.8	
$CdCl_2$	08-Jul-02	0	0.291	7.49	7.87	
$CdCl_2$	08-Jul-02	0	0.412	7.34	8.02	
$CdCl_2$	08-Jul-02	0	0.582	7.19	8.35	
$CdCl_2$	08-Jul-02	0	0.823	7.17	8.3	
$CdCl_2$	08-Jul-02	0	1.164	7.17	8.3	
$CdCl_2$	12-Jul-02	96	c	6.27		
$CdCl_2$	12-Jul-02	96	0.206	6.4		
$CdCl_2$	12-Jul-02	96	0.291	6.2		
$CdCl_2$	12-Jul-02	96	0.412	6.3		
$CdCl_2$	12-Jul-02	96	0.582	6.06		
$CdCl_2$	12-Jul-02	96	0.823	6.2		
$CdCl_2$	12-Jul-02	96	1.164	6.21		
DDT	23-Jun-03	0	c	8.4	7.9	
DDT	23-Jun-03	0	sc	7.4	8	
DDT	23-Jun-03	0	0.28	7.9	7.9	
DDT	23-Jun-03	0	4.5	7.91	8	
PCP	27-Jan-02	0	c	7.27	7.49	
PCP	27-Jan-02	0	sc	7.41	7.84	
PCP	27-Jan-02	0	0.32	7.39	7.12	
PCP	27-Jan-02	0	2	7.48	6.49	
TBT-Cl	27-Feb-03	0	c	8.04	8.17	21
TBT-Cl	27-Feb-03	0	sc	7.8	8.53	20.1
TBT-Cl	27-Feb-03	0	0.005	8.06	8.5	20.1
TBT-Cl	27-Feb-03	0	0.02	7.94	8.51	20.1
TNT	09-Dec-02	0	c	7.42	8.2	
TNT	09-Dec-02	0	7.07	7.53	8.01	
TNT	09-Dec-02	0	40.000	7.21	7.86	

Table 7.2: Physical and chemical variables of acute toxicity tests with *C. riparius*

chemical	date	time [h]	concentration [mgl^{-1}]	oxygen [mgl^{-1}]	pH
2,4-DCP	15-Jul-02	0	control	8.2	7.13
2,4-DCP	15-Jul-02	0	1.333	8.3	7.26
2,4-DCP	15-Jul-02	0	2	8.18	7.14
2,4-DCP	15-Jul-02	0	3	8.15	7.13
2,4-DCP	15-Jul-02	0	4.5	8.21	7.05
2,4-DCP	15-Jul-02	0	6.75		7.03
2,4-DCP	15-Jul-02	0	10.125	8.2	7.02
2,4-DCP	17-Jul-02	48	control		7.12
2,4-DCP	17-Jul-02	48	1.333		7.21
2,4-DCP	17-Jul-02	48	2		7.14
2,4-DCP	17-Jul-02	48	3		7.13
2,4-DCP	17-Jul-02	48	4.5		7.14
2,4-DCP	17-Jul-02	48	6.75		7.12
2,4-DCP	17-Jul-02	48	10.125		7.13
3,4-DCA	19-Feb-02	0	control	7.92	7.15
3,4-DCA	19-Feb-02	0	2.5	7.86	7.34
3,4-DCA	19-Feb-02	0	10	8.09	7.15
3,4-DCA	19-Feb-02	0	40	8.24	7.25
$CdCl_2$	04-Feb-02	0	control	8.21	7.04
$CdCl_2$	04-Feb-02	0	3.125	8.1	7.3
$CdCl_2$	04-Feb-02	0	12.5	7.86	7.08
$CdCl_2$	04-Feb-02	0	100	8.2	7.09
DDT	02-Jul-03	0	control	8.02	7.12
DDT	02-Jul-03	0	solvent control	8.1	7.15
DDT	02-Jul-03	0	0.00283	8.01	7.05
DDT	02-Jul-03	0	0.00917	7.94	7.16
DDT	02-Jul-03	0	0.0535	7.91	7.12
PCP	28-Aug-02	0	control	8.08	7.3

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chemical	date	time [h]	concentration [mg l^{-1}]	oxygen [mg l^{-1}]	pH
PCP	28-Aug-02	0	solvent control	7.92	7.15
PCP	28-Aug-02	0	0.25	8.1	7.15
PCP	28-Aug-02	0	0.5	8.05	7.14
PCP	28-Aug-02	0	1	8.15	7.08
PCP	28-Aug-02	0	2	8.21	7.09
PCP	28-Aug-02	0	4	7.98	7.02
PCP	30-Aug-02	48	control		7.21
PCP	30-Aug-02	48	solvent control		7.22
PCP	30-Aug-02	48	0.25		7.5
PCP	30-Aug-02	48	0.5		7.29
PCP	30-Aug-02	48	1		7.24
PCP	30-Aug-02	48	2		7.22
PCP	30-Aug-02	48	4		7.2
TBT-Cl	24-Jul-02	0	control	7.86	7.22
TBT-Cl	24-Jul-02	0	solvent control	8	7.23
TBT-Cl	24-Jul-02	0	0.0025		7.27
TBT-Cl	24-Jul-02	0	0.003536		7.19
TBT-Cl	24-Jul-02	0	0.005		7.17
TBT-Cl	24-Jul-02	0	0.00707		7.17
TBT-Cl	24-Jul-02	0	0.01		7.14
TBT-Cl	24-Jul-02	0	0.014124		7.12
TBT-Cl	24-Jul-02	0	0.02		7.13
TNT	04-Dec-02	0	control	8.06	7.21
TNT	04-Dec-02	0	solvent control	8.25	7.05
TNT	04-Dec-02	0	7.07	7.98	7.2
TNT	04-Dec-02	0	14.14	8.17	7.14
TNT	04-Dec-02	0	40	8.03	7.14

Table 7.3: Physical and chemical variables of sediment toxicity tests with *L. variegatus*

chemical	test number	date	concentration [mg kg^{-1}]	time [d]	rep	pH	O_2 [mg l^{-1}]	γ [μS cm^{-1}]	T [°C]
2,4-DCP	43	10-Oct-03	0	c	1	8.2	5.33	657	20.2
2,4-DCP	43	10-Oct-03	0	sc	1	8.31	5.4	660	20.3
2,4-DCP	43	10-Oct-03	0	1.6	1	8.28	5.16	649	20.3
2,4-DCP	43	10-Oct-03	0	8	1	8.42	5.98	590	20.3
2,4-DCP	43	10-Oct-03	0	40	1	8.43	5.85	659	20.2
2,4-DCP	43	10-Oct-03	0	200	1	8.47	5.88	658	20.3
2,4-DCP	43	10-Oct-03	0	1000	1	8.41	5.97	671	20.3
2,4-DCP	43	07-Nov-03	28	c	1	7.12	7.47	760	20.4
2,4-DCP	43	07-Nov-03	28	sc	1	8.31	7.85	646	20.3
2,4-DCP	43	07-Nov-03	28	1.6	1	8.27	7.02	680	20.2
2,4-DCP	43	07-Nov-03	28	8	1	8.18	6.51	614	20.2
2,4-DCP	43	07-Nov-03	28	40	1	8.29	7.63	681	20.1
2,4-DCP	43	07-Nov-03	28	200	1	8.27	7.18	676	20.1
2,4-DCP	43	07-Nov-03	28	1000	1	8.41	7.94	647	20.3
B(a)p	42	19-May-04	0	c	1	8.28	9.23	687	20.2
B(a)p	42	19-May-04	0	sc	1	8.51	9.42	681	20.2
B(a)p	42	19-May-04	0	0.064	1	8.37	9.18	659	20.2
B(a)p	42	19-May-04	0	0.064	2	8.4	8.37	685	20.2
B(a)p	42	19-May-04	0	0.064	3	8.44	9.09	693	20.3
B(a)p	42	19-May-04	0	0.32	1	8.44	9.5	694	20.3
B(a)p	42	19-May-04	0	1.6	1	8.53	9.7	694	20.3
B(a)p	42	19-May-04	0	8	1	8.47	9.63	692	20.3
B(a)p	42	19-May-04	0	8	2	8.52	9.6	693	20.4
B(a)p	42	19-May-04	0	8	3	8.47	9.58	685	20.4
B(a)p	42	19-May-04	0	40	1	8.6	9.64	687	20.3
B(a)p	42	19-May-04	0	200	1	8.64	9.86	680	20.4
B(a)p	42	19-May-04	0	1000	1	8.47	9.31	692	20.4
B(a)p	42	19-May-04	0	1000	2	8.54	9.64	688	20.4
B(a)p	42	19-May-04	0	1000	3	8.61	9.83	707	20.3
B(a)p	42	16-Jun-04	28	c	1	8.45	7.45	658	20.7
B(a)p	42	16-Jun-04	28	sc	1	7.94	5.72	600	20.7
B(a)p	42	16-Jun-04	28	0.064	1	8.39	7.29	606	20.6
B(a)p	42	16-Jun-04	28	0.064	2	8.19	6.87	609	20.7
B(a)p	42	16-Jun-04	28	0.32	1	8.33	7.19	605	20.7
B(a)p	42	16-Jun-04	28	1.6	1	8.35	6.65	591	20.6
B(a)p	42	16-Jun-04	28	8	1	8.53	7.65	570	20.7
B(a)p	42	16-Jun-04	28	8	2	8.32	7.3	580	20.7
B(a)p	42	16-Jun-04	28	40	1	8.34	7.28	566	20.8
B(a)p	42	16-Jun-04	28	200	1	8.13	6.16	627	20.8
B(a)p	42	16-Jun-04	28	1000	1	8.44	7.32	599	20.8
B(a)p	42	16-Jun-04	28	1000	2	8.48	7.49	642	20.8
$CdCl_2$	41	13-May-04	0	c	1	8.31	8.34	658	20.3
$CdCl_2$	41	13-May-04	0	sc	1	8.45	8.35	669	20.3
$CdCl_2$	41	13-May-04	0	0.0128	1	8.23	8.11	660	20.3
$CdCl_2$	41	13-May-04	0	0.0128	2	8.23	8.28	657	20.3
$CdCl_2$	41	13-May-04	0	0.0128	3	8.6	9.31	671	20.3
$CdCl_2$	41	13-May-04	0	0.064	1	8.5	8.9	670	20.3
$CdCl_2$	41	13-May-04	0	0.32	1	8.57	9.11	702	20.7
$CdCl_2$	41	13-May-04	0	1.6	1	8.64	8.18	680	20.5
$CdCl_2$	41	13-May-04	0	1.6	2	8.45	8.54	684	20.4
$CdCl_2$	41	13-May-04	0	1.6	3	8.6	9.38	677	20.5
$CdCl_2$	41	13-May-04	0	8	1	8.43	9.13	688	20.4
$CdCl_2$	41	13-May-04	0	40	1	8.59	9.71	675	20.4
$CdCl_2$	41	13-May-04	0	200	1	8.71	9.8	690	20.5
$CdCl_2$	41	13-May-04	0	200	2	8.73	9.9	682	20.5
$CdCl_2$	41	13-May-04	0	200	3	8.64	9.8	651	20.5

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chemical	test number	date	concentration [mg kg^{-1}]	time [d]	rep	pH	O_2 [mg l^{-1}]	γ [μS cm^{-1}]	T [°C]
$CdCl_2$	41	10-Jun-04	28	c	1	8.27	7.08	631	21
$CdCl_2$	41	10-Jun-04	28	sc	1	8.28	6.75	666	21
$CdCl_2$	41	10-Jun-04	28	0.0128	1	8.37	7.21	638	21
$CdCl_2$	41	10-Jun-04	28	0.0128	2	8.35	7.05	689	21
$CdCl_2$	41	10-Jun-04	28	0.064	1	8.37	7.06	683	20.9
$CdCl_2$	41	10-Jun-04	28	0.32	1	8.45	7.44	642	20.8
$CdCl_2$	41	10-Jun-04	28	1.6	1	8.46	7.54	676	20.8
$CdCl_2$	41	10-Jun-04	28	1.6	2	8.42	7.37	669	20.8
$CdCl_2$	41	10-Jun-04	28	8	1	8.26	7.03	637	20.8
$CdCl_2$	41	10-Jun-04	28	40	1	8.3	7.36	650	20.8
$CdCl_2$	41	10-Jun-04	28	200	1	8.62	8.21	648	20.8
$CdCl_2$	41	10-Jun-04	28	200	2	8.63	8.24	636	20.8
DDT	38	09-Mar-04	0	c	1	8.38	8.83	671	20.1
DDT	38	09-Mar-04	0	c	2	8.49	9.19	661	20
DDT	38	09-Mar-04	0	c	3	8.33	8.38	674	19.9
DDT	38	09-Mar-04	0	c	4	8.32	8.4	684	19.3
DDT	38	09-Mar-04	0	c	5	8.4	8.5	685	19.9
DDT	38	09-Mar-04	0	sc	1	8.4	8.4	685	20.1
DDT	38	09-Mar-04	0	sc	2	8.4	7.91	703	20.1
DDT	38	09-Mar-04	0	sc	3	8.35	7.55	673	20
DDT	38	09-Mar-04	0	sc	4	8.35	7.5	683	20.2
DDT	38	09-Mar-04	0	sc	5	8.36	7.4	650	20
DDT	38	09-Mar-04	0	sc	6	8.33	7.22	694	20
DDT	38	09-Mar-04	0	sc	7	8.58	7.81	698	19.9
DDT	38	09-Mar-04	0	0.2	1	8.21	7.34	693	20.2
DDT	38	09-Mar-04	0	0.2	2	8.38	9.05	696	19.5
DDT	38	09-Mar-04	0	0.2	3	8.33	6.38	706	19.8
DDT	38	09-Mar-04	0	0.2	4	8.17	6.97	675	19.7
DDT	38	09-Mar-04	0	0.2	5	8.31	7.08	688	20
DDT	38	09-Mar-04	0	0.2	6	8.44	7.05	733	19.7
DDT	38	09-Mar-04	0	0.2	7	8.46	7.33	668	20
DDT	38	09-Mar-04	0	1.41	1	8.53	7.8	661	20.1
DDT	38	09-Mar-04	0	1.41	2	8.55	7.79	683	20
DDT	38	09-Mar-04	0	1.41	3	8.26	7.1	696	19.9
DDT	38	09-Mar-04	0	1.41	4	8.39	7.14	685	19.9
DDT	38	09-Mar-04	0	1.41	5	8.5	7.38	677	19.8
DDT	38	09-Mar-04	0	1.41	6	8.45	7.33	657	20
DDT	38	09-Mar-04	0	10	1	8.46	7.71	691	20.2
DDT	38	09-Mar-04	0	10	2	8.45	7.62	684	20
DDT	38	09-Mar-04	0	10	3	8.4	7.23	676	20
DDT	38	09-Mar-04	0	10	4	8.54	7.85	705	20
DDT	38	09-Mar-04	0	10	5	8.5	7.55	803	19.9
DDT	38	09-Mar-04	0	10	6	8.29	6.94	761	20
DDT	38	09-Mar-04	0	70.71	1	8.39	7.4	685	20.1
DDT	38	09-Mar-04	0	70.71	2	8.42	6.65	757	20.1
DDT	38	09-Mar-04	0	70.71	3	8.26	6.87	639	20.1
DDT	38	09-Mar-04	0	70.71	4	8.33	6.52	618	20
DDT	38	09-Mar-04	0	70.71	5	8.33	8.56	718	20
DDT	38	09-Mar-04	0	70.71	6	8.53	8.22	649	19.9
DDT	38	09-Mar-04	0	500	1	8.39	7.73	630	19.9
DDT	38	09-Mar-04	0	500	2	8.35	7.23	702	20
DDT	38	09-Mar-04	0	500	3	8.38	7.59	721	19.9
DDT	38	09-Mar-04	0	500	4	8.39	7.05	712	20
DDT	38	09-Mar-04	0	500	5	8.48	7.7	705	19.9
DDT	38	09-Mar-04	0	500	6	8.43	7.62	682	19.7
DDT	38	09-Mar-04	0	500	7	8.43	7.3	701	19.8
DDT	38	06-Apr-04	28	c	1	8.47	7.17	557	20.4
DDT	38	06-Apr-04	28	c	2	8.33	7.13	600	20.5
DDT	38	06-Apr-04	28	c	3	8.34	6.53	634	20.2
DDT	38	06-Apr-04	28	c	4	8.34	6.51	664	20.2
DDT	38	06-Apr-04	28	c	5	8.36	6.17	609	20.2
DDT	38	06-Apr-04	28	sc	1	8.44	7.1	648	20.2
DDT	38	06-Apr-04	28	sc	2	8.46	7.4	612	19.8
DDT	38	06-Apr-04	28	sc	3	8.51	7.49	604	19.9
DDT	38	06-Apr-04	28	sc	4	8.56	7.22	593	20
DDT	38	06-Apr-04	28	sc	5	8.51	7.36	622	20
DDT	38	06-Apr-04	28	sc	6	8.48	7.03	620	20
DDT	38	06-Apr-04	28	0.2	1	8.42	7.01	614	20.1
DDT	38	06-Apr-04	28	0.2	2	8.45	7.11	625	20.2
DDT	38	06-Apr-04	28	0.2	3	8.44	6.95	590	19.9
DDT	38	06-Apr-04	28	0.2	4	8.59	7.31	652	19.8
DDT	38	06-Apr-04	28	0.2	5	8.59	7.45	579	19.9
DDT	38	06-Apr-04	28	0.2	6	8.64	7.55	620	19.9
DDT	38	06-Apr-04	28	1.41	1	8.49	7.22	643	20.4
DDT	38	06-Apr-04	28	1.41	2	8.56	7.53	603	20.1
DDT	38	06-Apr-04	28	1.41	3	8.48	7.28	631	19.8
DDT	38	06-Apr-04	28	1.41	4	8.34	6.61	598	19.7
DDT	38	06-Apr-04	28	1.41	5	8.67	7.59	611	19.6
DDT	38	06-Apr-04	28	10	1	8.33	7.11	591	20.2
DDT	38	06-Apr-04	28	10	2	8.38	6.89	583	20.2
DDT	38	06-Apr-04	28	10	3	8.46	7.15	632	20
DDT	38	06-Apr-04	28	10	4	8.42	6.02	717	19.9
DDT	38	06-Apr-04	28	10	5	8.33	6.72	653	19.8
DDT	38	06-Apr-04	28	70.71	1	8.55	6.49	689	20.9
DDT	38	06-Apr-04	28	70.71	2	8.48	6.95	545	20.7
DDT	38	06-Apr-04	28	70.71	3	8.25	6.5	546	20.6
DDT	38	06-Apr-04	28	70.71	4	8.53	6.6	644	20.7
DDT	38	06-Apr-04	28	70.71	5	8.48	6.99	576	20.6
DDT	38	06-Apr-04	28	500	1	8.38	6.62	614	20.2
DDT	38	06-Apr-04	28	500	2	8.46	7.03	630	20.3

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chemical	test number	date	concentration [mg kg^{-1}]	time [d]	rep	pH	O_2 [mg l^{-1}]	γ [μS cm^{-1}]	T [°C]
DDT	38	06-Apr-04	28	500	3	8.51	7.17	572	20.3
DDT	38	06-Apr-04	28	500	4	8.48	7.03	566	20
DDT	38	06-Apr-04	28	500	5	8.54	7.19	621	20
DDT	38	06-Apr-04	28	500	6	8.49	6.75	652	20.1
PCP	44	10-Oct-03	0	c	1	8.2	5.33	657	20.2
PCP	44	10-Oct-03	0	sc	1	8.31	5.4	660	20.3
PCP	44	10-Oct-03	0	0.064	1	8.19	4.78	618	20.3
PCP	44	10-Oct-03	0	0.32	1	8.41	5.42	694	20.2
PCP	44	10-Oct-03	0	1.6	1	8.43	5.58	650	20.2
PCP	44	10-Oct-03	0	8	1	8.42	4.83	652	20.3
PCP	44	10-Oct-03	0	40	1	8.6	5.91	666	20.2
PCP	44	10-Oct-03	0	200	1	8.67	6.26	604	20.2
PCP	44	10-Oct-03	0	1000	1	8.51	6.3	591	20.3
PCP	44	07-Nov-03	28	0.064	1	8.24	6.62	687	20.2
PCP	44	07-Nov-03	28	0.32	1	8.19	6.55	632	20.1
PCP	44	07-Nov-03	28	1.6	1	8.2	6.58	630	20.1
PCP	44	07-Nov-03	28	8	1	8.21	7.97	641	20.1
PCP	44	07-Nov-03	28	40	1	8.3	7.65	631	20
PCP	44	07-Nov-03	28	200	1	8.42	8.32	614	20.1
PCP	44	07-Nov-03	28	1000	1	8.48	8.53	625	20.1
PCP	45 *	06-Nov-03	0	c	1	7.94	7.54	904	19.7
PCP	45 *	06-Nov-03	0	c	2	8.18	7.66	920	19.8
PCP	45 *	06-Nov-03	0	c	3	8.3	7.93	914	19.7
PCP	45 *	06-Nov-03	0	c	4	8.2	7.57	907	19.8
PCP	45 *	06-Nov-03	0	c	5	8.19	7.52	910	19.9
PCP	45 *	06-Nov-03	0	c	6	8.04	6.96	912	19.9
PCP	45 *	06-Nov-03	0	c	7	8.16	7.78	891	19.6
PCP	45 *	06-Nov-03	0	c	8	8.19	7.98	884	19.6
PCP	45 *	06-Nov-03	0	c	9	8.02	7.65	888	19.6
PCP	45 *	06-Nov-03	0	c	10	8.21	8.03	886	19.6
PCP	45 *	06-Nov-03	0	sc	1	8	7.45	907	20.1
PCP	45 *	06-Nov-03	0	sc	2	8.09	7.33	907	20.1
PCP	45 *	06-Nov-03	0	sc	3	7.9	7.12	911	20
PCP	45 *	06-Nov-03	0	0.05	1	7.92	7.22	913	19.9
PCP	45 *	06-Nov-03	0	0.05	2	8.07	7.42	955	19.9
PCP	45 *	06-Nov-03	0	0.05	3	8.06	7.3	930	20
PCP	45 *	06-Nov-03	0	0.05	4	8.13	7.92	916	19.8
PCP	45 *	06-Nov-03	0	0.05	5	8.15	7.76	918	19.8
PCP	45 *	06-Nov-03	0	0.05	6	8.16	8.05	922	19.8
PCP	45 *	06-Nov-03	0	0.05	7	8.24	8	930	19.6
PCP	45 *	06-Nov-03	0	0.25	1	8.09	7.48	932	20
PCP	45 *	06-Nov-03	0	0.25	2	8.29	7.85	992	19.9
PCP	45 *	06-Nov-03	0	0.25	3	8.34	8.05	928	19.9
PCP	45 *	06-Nov-03	0	1.25	1	8	7.57	916	19.9
PCP	45 *	06-Nov-03	0	1.25	2	8.15	7.34	926	19.9
PCP	45 *	06-Nov-03	0	1.25	3	8.22	7.5	924	19.9
PCP	45 *	06-Nov-03	0	1.25	4	8.22	8	901	19.7
PCP	45 *	06-Nov-03	0	1.25	5	8.21	7.56	902	19.7
PCP	45 *	06-Nov-03	0	1.25	6	7.89	6.01	929	19.7
PCP	45 *	06-Nov-03	0	1.25	7	8.28	7.4	924	19.7
PCP	45 *	06-Nov-03	0	6.25	1	8.32	8.2	938	19.8
PCP	45 *	06-Nov-03	0	6.25	2	8.25	7.68	926	19.8
PCP	45 *	06-Nov-03	0	6.25	3	8.21	7.5	920	19.8
PCP	45 *	06-Nov-03	0	31.25	1	8.13	7.71	889	19.9
PCP	45 *	06-Nov-03	0	31.25	2	8.24	8.07	954	19.6
PCP	45 *	06-Nov-03	0	31.25	3	8.25	7.95	896	19.8
PCP	45 *	06-Nov-03	0	31.25	4	8.16	7.9	890	19.7
PCP	45 *	06-Nov-03	0	31.25	5	8.24	8.15	885	19.7
PCP	45 *	06-Nov-03	0	31.25	6	8.22	7.97	869	19.7
PCP	45 *	06-Nov-03	0	31.25	7	8.21	7.77	888	19.7
PCP	45 *	12-Nov-03	6	c	5	8.2	7.46	995	20.2
PCP	45 *	12-Nov-03	6	1.25	3	8.27	7.3	1004	20.1
PCP	45 *	12-Nov-03	6	31.25	3	8.27	7.5	984	20
PCP	45 *	17-Nov-03	11	c	5	8.01	6.44	1052	19.3
PCP	45 *	17-Nov-03	11	1.25	3	8.02	6.23	1059	18.9
PCP	45 *	17-Nov-03	11	31.25	3	8.08	6.11	1047	19.1
PCP	45 *	20-Nov-03	14	c	5	8.41	6.44	1101	20
PCP	45 *	20-Nov-03	14	1.25	3	8.43	6.61	1049	19.8
PCP	45 *	20-Nov-03	14	31.25	3	8.52	6.25	1045	19.7
PCP	45 *	21-Nov-03	15	c	5	8.55	8.86	1164	19.9
PCP	45 *	21-Nov-03	15	1.25	3	8.43	8.71	1056	19.9
PCP	45 *	21-Nov-03	15	31.25	3	8.47	8.92	1048	19.7
PCP	45 *	24-Nov-03	18	c	5	8.23	7.32	1131	19.7
PCP	45 *	24-Nov-03	18	1.25	3	8.51	7.78	1037	19.6
PCP	45 *	24-Nov-03	18	31.25	3	8.32	7.36	1019	19.6
PCP	45 *	26-Nov-03	20	c	5	8.29	7.88	1111	17.9
PCP	45 *	26-Nov-03	20	1.25	3	8.44	7.91	1047	18
PCP	45 *	26-Nov-03	20	31.25	3	8.42	7.91	1077	18.2
PCP	45 *	28-Nov-03	22	c	5	8.33	7.22	1095	20.8
PCP	45 *	28-Nov-03	22	1.25	3	8.45	7.27	1041	20.9
PCP	45 *	28-Nov-03	22	31.25	3	8.52	7.45	1017	20.9
PCP	45 *	01-Dec-03	25	c	5	8.09	7.19	1088	20.5
PCP	45 *	01-Dec-03	25	1.25	3	8.22	7.12	1004	20.6
PCP	45 *	01-Dec-03	25	31.25	3	8.29	7.23	1022	20.6
PCP	45 *	04-Dec-03	28	c	1	8.31	7.6	955	19.6
PCP	45 *	04-Dec-03	28	c	2	8.29	6.8	969	20.1
PCP	45 *	04-Dec-03	28	c	3	8.25	7.15	952	20.2
PCP	45 *	04-Dec-03	28	c	4	8.26	6.74	951	20.1
PCP	45 *	04-Dec-03	28	c	5	8.36	7.1	1082	20.1
PCP	45 *	04-Dec-03	28	c	6	8.52	7.5	997	20.3

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chemical	test number	date	concentration [mg kg^{-1}]	time [d]	rep	pH	O_2 [mg l^{-1}]	γ [μS cm^{-1}]	T [°C]
PCP	45 *	04-Dec-03	28	c	8	8.11	7.74	1013	20.1
PCP	45 *	04-Dec-03	28	c	10	7.89	7.43	944	20.1
PCP	45 *	04-Dec-03	28	sc	1	8.43	7.15	932	20.4
PCP	45 *	04-Dec-03	28	sc	2	8.34	7	1008	20.4
PCP	45 *	04-Dec-03	28	sc	3	8.29	7.2	935	20.4
PCP	45 *	04-Dec-03	28	0.05	1	8.15	6.9	1033	20.3
PCP	45 *	04-Dec-03	28	0.05	2	8.29	7.04	972	20.5
PCP	45 *	04-Dec-03	28	0.05	3	8.37	7.07	1029	20.5
PCP	45 *	04-Dec-03	28	0.05	4	7.94	7.61	969	20
PCP	45 *	04-Dec-03	28	0.05	5	8.1	7.75	1030	20.2
PCP	45 *	04-Dec-03	28	0.25	1	8.48	7.38	1013	20.5
PCP	45 *	04-Dec-03	28	0.25	2	8.41	6.54	1090	20.4
PCP	45 *	04-Dec-03	28	0.25	3	8.51	7.3	1014	20.5
PCP	45 *	04-Dec-03	28	1.25	1	8.37	6.8	956	20.7
PCP	45 *	04-Dec-03	28	1.25	2	8.33	6.5	943	20.7
PCP	45 *	04-Dec-03	28	1.25	3	8.48	7.3	982	20.7
PCP	45 *	04-Dec-03	28	1.25	5	7.81	7.25	994	20.4
PCP	45 *	04-Dec-03	28	1.25	6	7.92	7.4	1042	20.3
PCP	45 *	04-Dec-03	28	6.25	1	8.25	7.27	972	20.4
PCP	45 *	04-Dec-03	28	6.25	2	8.42	7.25	978	20.6
PCP	45 *	04-Dec-03	28	6.25	3	8.42	7.36	957	20.6
PCP	45 *	04-Dec-03	28	31.25	1	8.49	6.94	1028	20.4
PCP	45 *	04-Dec-03	28	31.25	2	8.46	7.19	1101	20.5
PCP	45 *	04-Dec-03	28	31.25	3	8.45	6.76	1014	20.6
PCP	45 *	04-Dec-03	28	31.25	4	8.03	7.4	1037	20.2
PCP	45 *	04-Dec-03	28	31.25	6	8.06	7.47	1008	20.2
TBT-Cl	39	12-Mar-04	0	c	1	8.28	7.24	712	19.5
TBT-Cl	39	12-Mar-04	0	sc	1	8.36	7.36	697	19.4
TBT-Cl	39	12-Mar-04	0	0.0064	1	8.33	7.12	683	19.4
TBT-Cl	39	12-Mar-04	0	0.0064	2	8.17	6.29	687	19.3
TBT-Cl	39	12-Mar-04	0	0.0064	3	8.07	5.6	693	19.3
TBT-Cl	39	12-Mar-04	0	0.032	1	8.27	7.05	683	18.7
TBT-Cl	39	12-Mar-04	0	0.16	1	8.41	7.52	704	18.6
TBT-Cl	39	12-Mar-04	0	0.8	1	8.43	7.51	696	18.4
TBT-Cl	39	12-Mar-04	0	0.8	2	8.42	7.2	696	18.6
TBT-Cl	39	12-Mar-04	0	0.8	3	8.5	7.56	687	18.5
TBT-Cl	39	12-Mar-04	0	4	1	8.55	7.16	1021	18.7
TBT-Cl	39	12-Mar-04	0	20	1	8.41	7.14	678	18.7
TBT-Cl	39	12-Mar-04	0	100	1	8.59	8.28	643	18.6
TBT-Cl	39	12-Mar-04	0	100	2	8.6	8.15	635	18.5
TBT-Cl	39	12-Mar-04	0	100	3	8.63	8.2	640	18.4
TBT-Cl	39	08-Apr-04	27	c	1	8.11	7.41	561	18.4
TBT-Cl	39	08-Apr-04	27	sc	1	8.32	7.14	605	18.7
TBT-Cl	39	08-Apr-04	27	0.0064	1	8.3	7.09	560	18
TBT-Cl	39	08-Apr-04	27	0.0064	2	8.21	6.55		20.4
TBT-Cl	39	08-Apr-04	27	0.032	1	8.32	7.27	574	19
TBT-Cl	39	08-Apr-04	27	0.16	1	8.27	6.73	615	19.5
TBT-Cl	39	08-Apr-04	27	0.8	1	8.22	6.59	597	20
TBT-Cl	39	08-Apr-04	27	0.8	2	8.32	6.62		20.7
TBT-Cl	39	08-Apr-04	27	4	1	8.32	6.4	906	19.3
TBT-Cl	39	08-Apr-04	27	20	1	8.34	6.82	597	20.7
TBT-Cl	39	08-Apr-04	27	100	1	8.36	7.12	588	20.5
TBT-Cl	39	08-Apr-04	27	100	2	8.49	6.74		20.5
TNT	40	18-Mar-04	0	c	1	8.16	7.1	677	19.1
TNT	40	18-Mar-04	0	sc	1	8.22	7.07	717	19.2
TNT	40	18-Mar-04	0	0.032	1	8	6	684	19.3
TNT	40	18-Mar-04	0	0.032	2	8.22	6.3	683	19.4
TNT	40	18-Mar-04	0	0.032	3	7.97	5.63	671	19.3
TNT	40	18-Mar-04	0	0.16	1	8.22	6.86	688	19.4
TNT	40	18-Mar-04	0	0.8	1	8.21	6.34	700	19.5
TNT	40	18-Mar-04	0	4	1	8.26	6.05	799	19.4
TNT	40	18-Mar-04	0	4	2	8.29	6.53	683	19.4
TNT	40	18-Mar-04	0	4	3	8.3	6.55	679	19.5
TNT	40	18-Mar-04	0	20	1	8.17	7	676	19.5
TNT	40	18-Mar-04	0	100	1	8.4	6.83	668	19.4
TNT	40	18-Mar-04	0	500	1	8.54	7.28	636	19.4
TNT	40	18-Mar-04	0	500	2	8.43	6.91	648	19.5
TNT	40	18-Mar-04	0	500	3	8.3	6.75	657	19.5
TNT	40	14-Apr-04	27	c	1	8.07	8.52	598	18.8
TNT	40	14-Apr-04	27	sc	1	8.03	7.03	566	18.6
TNT	40	14-Apr-04	27	0.032	1	8.31	6.64	682	18.6
TNT	40	14-Apr-04	27	0.032	2	8.22	6.86	583	18.7
TNT	40	14-Apr-04	27	0.16	1	8.23	6.7	587	18.9
TNT	40	14-Apr-04	27	0.8	1	8.36	5.36	628	19.2
TNT	40	14-Apr-04	27	4	1	8.53	6.75	579	19.2
TNT	40	14-Apr-04	27	4	2	8.03	5.77	621	19
TNT	40	14-Apr-04	27	20	1	8.1	6.03	584	19
TNT	40	14-Apr-04	27	100	1	7.96	5.56	593	19.1
TNT	40	14-Apr-04	27	500	1	8.07	6.48	607	19.1
TNT	40	14-Apr-04	27	500	2	8.39	7.46	573	19.2

45 * = PCP sediment toxicity test of the international ring test funded by German Federal Environmental Agency

Table 7.4: Physical and chemical variables of sediment toxicity tests with *C. riparius* at beginning (t0d) and end of the exposure(t28d)

Date	test number	chemical	concentration [mg kg^{-1}]	time [d]	rep	pH	O_2 [mg l^{-1}]	γ [μS cm^{-1}]	T [°C]
05-Nov-03	9	2,4-DCP	control	0	1	8.07	6.59	976	20.5
05-Nov-03	9	2,4-DCP	control	0	2	8.14	6.55	957	20.6
05-Nov-03	9	2,4-DCP	control	0	3	8.13	6.28	1008	20.5
05-Nov-03	9	2,4-DCP	control	0	4	8.14	6.54	992	20.5
05-Nov-03	9	2,4-DCP	control	0	5	8.17	6.73	950	20.4
05-Nov-03	9	2,4-DCP	solvent control	0	1	8.13	6.5	917	20.6
05-Nov-03	9	2,4-DCP	solvent control	0	2	8.2	6.87	991	20.5

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Date	test number	chemical	concentration [mg kg^{-1}]	time [d]	rep	pH	O_2 [mg l^{-1}]	γ [μS cm^{-1}]	T [°C]
05-Nov-03	9	2,4-DCP	solvent control	0	3	8.22	6.78	948	20.5
05-Nov-03	9	2,4-DCP	solvent control	0	4	8.18	6.14	998	20.4
05-Nov-03	9	2,4-DCP	solvent control	0	5	8.23	6.76	943	20.4
05-Nov-03	9	2,4-DCP	8	0	1	8.06	5.98	970	20.6
05-Nov-03	9	2,4-DCP	8	0	2	8.21	6.84	1000	20.4
05-Nov-03	9	2,4-DCP	8	0	3	8.12	6.31	1006	20.4
05-Nov-03	9	2,4-DCP	8	0	4	8.16	6.62	991	20.5
05-Nov-03	9	2,4-DCP	8	0	5	8.16	6.55	995	20.7
05-Nov-03	9	2,4-DCP	8	0	6	8.1	5.96	995	20.8
05-Nov-03	9	2,4-DCP	17.89	0	1	8.06	6.09	964	20.9
05-Nov-03	9	2,4-DCP	17.89	0	2	8.19	6.18	983	21
05-Nov-03	9	2,4-DCP	17.89	0	3	8.18	6.15	963	20.9
05-Nov-03	9	2,4-DCP	17.89	0	4	8.17	6.4	979	20.9
05-Nov-03	9	2,4-DCP	17.89	0	5	8.16	6.15	1000	20.9
05-Nov-03	9	2,4-DCP	17.89	0	6	8.24	6.52	1007	21
05-Nov-03	9	2,4-DCP	40	0	1	7.95	5.05	1002	20.7
05-Nov-03	9	2,4-DCP	40	0	2	8.15	6.27	970	20.8
05-Nov-03	9	2,4-DCP	40	0	3	8.24	5.31	960	21
05-Nov-03	9	2,4-DCP	40	0	4	8.2	6.34	939	21
05-Nov-03	9	2,4-DCP	40	0	5	8.26	6.78	944	21
05-Nov-03	9	2,4-DCP	40	0	6	8.3	6.82	932	21
05-Nov-03	9	2,4-DCP	89.44	0	1	8.13	6.37	923	20.9
05-Nov-03	9	2,4-DCP	89.44	0	2	8.28	5.39	917	21.2
05-Nov-03	9	2,4-DCP	89.44	0	3	8.15	6.14	932	20.8
05-Nov-03	9	2,4-DCP	89.44	0	4	8.21	6.55	935	20.8
05-Nov-03	9	2,4-DCP	89.44	0	5	8.24	6.67	933	20.8
05-Nov-03	9	2,4-DCP	89.44	0	6	8.29	6.9	912	20.9
05-Nov-03	9	2,4-DCP	200	0	1	8.32	7.34	925	20.8
05-Nov-03	9	2,4-DCP	200	0	2	8.32	6.91	908	20.6
05-Nov-03	9	2,4-DCP	200	0	3	8.28	6.99	984	20.6
05-Nov-03	9	2,4-DCP	200	0	4	8.17	6.36	941	20.7
05-Nov-03	9	2,4-DCP	200	0	5	8.14	6.21	930	20.7
05-Nov-03	9	2,4-DCP	200	0	6	8.2	6.89	946	20.7
05-Dec-03	9	2,4-DCP	control	28	1	8.36	7.18	890	20.1
05-Dec-03	9	2,4-DCP	control	28	2	8.53	7.75	938	20.1
05-Dec-03	9	2,4-DCP	control	28	3	8.54	7.65	902	20.1
05-Dec-03	9	2,4-DCP	control	28	4	8.5	7.36	939	20.1
05-Dec-03	9	2,4-DCP	control	28	5	8.5	7.35	943	20.2
05-Dec-03	9	2,4-DCP	solvent control	28	1	8.5	7.59	895	20.1
05-Dec-03	9	2,4-DCP	solvent control	28	2	8.57	7.85	874	20.1
05-Dec-03	9	2,4-DCP	solvent control	28	3	8.62	7.89	934	20.3
05-Dec-03	9	2,4-DCP	solvent control	28	4	8.48	6.99	956	20.2
05-Dec-03	9	2,4-DCP	solvent control	28	5	8.53	7.75	968	19.9
05-Dec-03	9	2,4-DCP	8	28	1	8.39	7.22	992	20.2
05-Dec-03	9	2,4-DCP	8	28	2	8.5	7.33	1015	20.2
05-Dec-03	9	2,4-DCP	8	28	3	8.52	7.34	967	20.2
05-Dec-03	9	2,4-DCP	8	28	4	8.44	7.18	948	20.1
05-Dec-03	9	2,4-DCP	8	28	5	8.42	7.24	985	20.1
05-Dec-03	9	2,4-DCP	17.89	28	1	8.51	7.55	953	20.2
05-Dec-03	9	2,4-DCP	17.89	28	2	8.52	7.53	938	20.1
05-Dec-03	9	2,4-DCP	17.89	28	3	8.58	7.83	911	20.4
05-Dec-03	9	2,4-DCP	17.89	28	4	8.53	7.35	956	20.2
05-Dec-03	9	2,4-DCP	17.89	28	5	8.54	7.97	907	20.4
05-Dec-03	9	2,4-DCP	40	28	1	8.43	7.18	970	20.2
05-Dec-03	9	2,4-DCP	40	28	2	8.48	7.24	939	20.1
05-Dec-03	9	2,4-DCP	40	28	3	8.42	5.52	926	20.1
05-Dec-03	9	2,4-DCP	40	28	4	8.46	6.86	906	20.2
05-Dec-03	9	2,4-DCP	40	28	5	8.42	6.55	920	20.1
05-Dec-03	9	2,4-DCP	89.44	28	1	8.41	7.12	898	20
05-Dec-03	9	2,4-DCP	89.44	28	2	8.48	7.23	861	20.1
05-Dec-03	9	2,4-DCP	89.44	28	3	8.54	7.32	856	20.1
05-Dec-03	9	2,4-DCP	89.44	28	4	8.51	7.21	859	20
05-Dec-03	9	2,4-DCP	89.44	28	5	8.54	7.34	861	20
05-Dec-03	9	2,4-DCP	200	28	1	8.37	7.32	848	20.1
05-Dec-03	9	2,4-DCP	200	28	2	8.49	7.52	897	20.1
05-Dec-03	9	2,4-DCP	200	28	3	8.48	7.26	881	20.1
05-Dec-03	9	2,4-DCP	200	28	4	8.4	6.71	869	20
05-Dec-03	9	2,4-DCP	200	28	5	8.48	7.3	820	20.1
02-Jun-04	10	B(a)p	control	0	1	8.1	3.7	1044	19.2
02-Jun-04	10	B(a)p	control	0	2	8.25	5.7	1071	19.2
02-Jun-04	10	B(a)p	control	0	3	8.65	7.39	1013	19.5
02-Jun-04	10	B(a)p	control	0	4	8.4	6.04	1019	19.4
02-Jun-04	10	B(a)p	control	0	5	8.48	6.69	1031	19.3
02-Jun-04	10	B(a)p	solvent control	0	1	8.57	7.36	997	19
02-Jun-04	10	B(a)p	solvent control	0	2	8.43	6.48	1003	19.1
02-Jun-04	10	B(a)p	solvent control	0	3	8.65	7.79	965	19.3
02-Jun-04	10	B(a)p	solvent control	0	4	8.62	7.6	1000	19.2
02-Jun-04	10	B(a)p	solvent control	0	5	8.65	7.86	960	19.3
02-Jun-04	10	B(a)p	solvent control	0	6	7.04	8.53	1031	19.3
02-Jun-04	10	B(a)p	0.1	0	1	8.43	7.16	1020	19.4
02-Jun-04	10	B(a)p	0.1	0	2	8.24	6.4	994	19.5
02-Jun-04	10	B(a)p	0.1	0	3	8.33	6.82	1000	19.6
02-Jun-04	10	B(a)p	0.1	0	4	8.38	6.62	1029	19.4
02-Jun-04	10	B(a)p	0.1	0	5	8.36	7	1007	19.6
02-Jun-04	10	B(a)p	0.1	0	6	8.13	5.28	1010	19.5
02-Jun-04	10	B(a)p	1	0	1	8.19	6.65	1006	19.6
02-Jun-04	10	B(a)p	1	0	2	8.32	6.89	1033	19.6
02-Jun-04	10	B(a)p	1	0	3	8.34	7	947	19.7
02-Jun-04	10	B(a)p	1	0	4	8.42	7.13	1017	19.6
02-Jun-04	10	B(a)p	1	0	5	8.14	5.74	964	19.5

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Date	test number	chemical	concentration [mg kg^{-1}]	time [d]	rep	pH	O_2 [mg l^{-1}]	γ [μS cm^{-1}]	T [°C]
02-Jun-04	10	B(a)p	1	0	6	8.52	7.39	967	19.5
02-Jun-04	10	B(a)p	10	0	1	8.45	7.07	1001	19.6
02-Jun-04	10	B(a)p	10	0	2	8.18	6.18	1018	19.6
02-Jun-04	10	B(a)p	10	0	3	8.09	5.2	1023	19.8
02-Jun-04	10	B(a)p	10	0	4	8.33	6.71	1018	19.8
02-Jun-04	10	B(a)p	10	0	5	8.25	6.62	1042	19.8
02-Jun-04	10	B(a)p	10	0	6	8.2	6.61	1060	19.7
02-Jun-04	10	B(a)p	100	0	1	8.38	6.92	1025	19.6
02-Jun-04	10	B(a)p	100	0	2	8.2	5.17	1030	19.6
02-Jun-04	10	B(a)p	100	0	3	8.26	6.47	999	19.7
02-Jun-04	10	B(a)p	100	0	4	8.59	7.52	1019	19.8
02-Jun-04	10	B(a)p	100	0	5	8.56	7.48	1007	19.7
02-Jun-04	10	B(a)p	100	0	6	8.14	5.78	1013	19.7
02-Jun-04	10	B(a)p	1000	0	1	8.43	6.98	1096	19.6
02-Jun-04	10	B(a)p	1000	0	2	8.73	7.89	1104	19.7
02-Jun-04	10	B(a)p	1000	0	3	8.26	6.44	1070	19.8
02-Jun-04	10	B(a)p	1000	0	4	8.43	6.65	1085	19.8
02-Jun-04	10	B(a)p	1000	0	5	8.64	7.64	1034	19.8
02-Jun-04	10	B(a)p	1000	0	6	8.63	7.78	1122	19.8
30-Jun-04	10	B(a)p	control	28	1	8.64	7.56	991	18.7
30-Jun-04	10	B(a)p	control	28	2	8.6	7.47	1048	18.7
30-Jun-04	10	B(a)p	control	28	3	8.72	8.01	954	18.8
30-Jun-04	10	B(a)p	control	28	4	8.76	7.9	992	18.8
30-Jun-04	10	B(a)p	control	28	5	8.7	7.55	992	18.8
30-Jun-04	10	B(a)p	solvent control	28	1	8.77	7.41	1039	19
30-Jun-04	10	B(a)p	solvent control	28	2	8.42	6.54	1007	19
30-Jun-04	10	B(a)p	solvent control	28	3	8.48	6.57	1010	19.1
30-Jun-04	10	B(a)p	solvent control	28	4	8.67	7.39	974	19.1
30-Jun-04	10	B(a)p	solvent control	28	5	8.69	7.62	971	19
30-Jun-04	10	B(a)p	solvent control	28	6	8.7	7.47	1005	18.9
30-Jun-04	10	B(a)p	0.1	28	1	8.66	6.84	1151	18.9
30-Jun-04	10	B(a)p	0.1	28	2	8.59	6.74	1191	18.8
30-Jun-04	10	B(a)p	0.1	28	3	8.56	6.57	1036	18.8
30-Jun-04	10	B(a)p	0.1	28	4	8.69	7.03	1053	18.8
30-Jun-04	10	B(a)p	0.1	28	5	8.55	6.68	1134	18.8
30-Jun-04	10	B(a)p	1	28	1	8.56	6.55	993	18.9
30-Jun-04	10	B(a)p	1	28	2	8.42	5.82	1055	19
30-Jun-04	10	B(a)p	1	28	3	8.51	6.45	1012	18.9
30-Jun-04	10	B(a)p	1	28	4	8.58	6.8	1016	18.9
30-Jun-04	10	B(a)p	1	28	5	8.58	6.84	970	18.8
30-Jun-04	10	B(a)p	10	28	1	8.53	5.63	1033	18.9
30-Jun-04	10	B(a)p	10	28	2	8.64	6.63	962	19.1
30-Jun-04	10	B(a)p	10	28	3	8.85	7.13	1036	19.1
30-Jun-04	10	B(a)p	10	28	4	8.48	5.91	1038	19.1
30-Jun-04	10	B(a)p	10	28	5	8.74	6.91	1016	19.1
30-Jun-04	10	B(a)p	100	28	1	8.61	6.64	994	19
30-Jun-04	10	B(a)p	100	28	2	8.75	7.1	1015	19.2
30-Jun-04	10	B(a)p	100	28	3	8.46	5.19	987	19.2
30-Jun-04	10	B(a)p	100	28	4	8.6	6.4	1008	19.2
30-Jun-04	10	B(a)p	100	28	5	8.57	6.45	1066	19.2
30-Jun-04	10	B(a)p	1000	28	1	8.77	7.08	993	19
30-Jun-04	10	B(a)p	1000	28	2	8.9	7.33	1062	19.2
30-Jun-04	10	B(a)p	1000	28	3	8.69	6.56	1089	19.3
30-Jun-04	10	B(a)p	1000	28	4	8.56	6.1	996	19.3
30-Jun-04	10	B(a)p	1000	28	5	8.66	6.69	1024	19.3
17-Jun-04	11	$CdCl_2$	control	0	1	8.68	7.46	988	20.1
17-Jun-04	11	$CdCl_2$	control	0	2	8.7	7.33	973	20.1
17-Jun-04	11	$CdCl_2$	control	0	3	8.69	7.46	946	20.2
17-Jun-04	11	$CdCl_2$	control	0	4	8.75	7.88	980	20.1
17-Jun-04	11	$CdCl_2$	control	0	5	8.69	7.46	988	20.1
17-Jun-04	11	$CdCl_2$	solvent control	0	1	8.56	7.31	958	20.2
17-Jun-04	11	$CdCl_2$	solvent control	0	2	8.48	7.06	943	20.2
17-Jun-04	11	$CdCl_2$	solvent control	0	3	8.71	7.7	915	20.2
17-Jun-04	11	$CdCl_2$	solvent control	0	4	8.63	7.78	951	20.2
17-Jun-04	11	$CdCl_2$	solvent control	0	5	8.78	7.67	907	20.2
17-Jun-04	11	$CdCl_2$	0.02	0	1	8.54	7.23	952	20.2
17-Jun-04	11	$CdCl_2$	0.02	0	2	8.65	7.51	917	20.1
17-Jun-04	11	$CdCl_2$	0.02	0	3	8.46	6.86	959	20.1
17-Jun-04	11	$CdCl_2$	0.02	0	4	8.67	7.15	927	20.2
17-Jun-04	11	$CdCl_2$	0.02	0	5	8.65	6.91	898	20.2
17-Jun-04	11	$CdCl_2$	0.02	0	6	8.64	7.29	932	20.2
17-Jun-04	11	$CdCl_2$	0.2	0	1	8.49	7.49	964	20
17-Jun-04	11	$CdCl_2$	0.2	0	2	8.57	6.98	941	20
17-Jun-04	11	$CdCl_2$	0.2	0	3	8.66	7.65	972	20
17-Jun-04	11	$CdCl_2$	0.2	0	4	8.72	7.83	950	20
17-Jun-04	11	$CdCl_2$	0.2	0	5	8.66	7.65	931	20
17-Jun-04	11	$CdCl_2$	0.2	0	6	8.5	7.08	975	20
17-Jun-04	11	$CdCl_2$	2	0	1	8.65	7.68	943	20
17-Jun-04	11	$CdCl_2$	2	0	2	8.64	7.37	915	20
17-Jun-04	11	$CdCl_2$	2	0	3	8.6	7.57	955	20
17-Jun-04	11	$CdCl_2$	2	0	4	8.58	7.57	934	20
17-Jun-04	11	$CdCl_2$	2	0	5	8.61	7.56	946	20
17-Jun-04	11	$CdCl_2$	2	0	6	8.53	7.25	964	20.1
17-Jun-04	11	$CdCl_2$	20	0	1	8.72	8.08	940	20
17-Jun-04	11	$CdCl_2$	20	0	2	8.78	8.04	944	20
17-Jun-04	11	$CdCl_2$	20	0	3	8.81	8.12	897	20
17-Jun-04	11	$CdCl_2$	20	0	4	8.63	7.87	915	20
17-Jun-04	11	$CdCl_2$	20	0	5	8.48	7.36	960	20
17-Jun-04	11	$CdCl_2$	20	0	6	8.56	7.5	943	20
17-Jun-04	11	$CdCl_2$	200	0	1	8.7	7.38	1023	20

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Date	test number	chemical	concentration [mg kg^{-1}]	time [d]	rep	pH	O_2 [mg l^{-1}]	γ [μS cm^{-1}]	T [°C]
17-Jun-04	11	$CdCl_2$	200	0	2	8.78	7.84	916	20
17-Jun-04	11	$CdCl_2$	200	0	3	8.8	8.01	1016	20
17-Jun-04	11	$CdCl_2$	200	0	4	8.67	7.97	918	20.1
17-Jun-04	11	$CdCl_2$	200	0	5	8.78	8.09	953	20
17-Jun-04	11	$CdCl_2$	200	0	6	8.67	7.76	952	20.1
14-Jul-04	11	$CdCl_2$	control	28	1	8.73	6.74	996	19.6
14-Jul-04	11	$CdCl_2$	control	28	2	8.59	6.43	1049	19.6
14-Jul-04	11	$CdCl_2$	control	28	3	8.64	7.13	978	19.6
14-Jul-04	11	$CdCl_2$	control	28	4	8.57	6.41	1000	19.6
14-Jul-04	11	$CdCl_2$	control	28	5	8.67	7.14	1032	19.7
14-Jul-04	11	$CdCl_2$	solvent control	28	1	8.62	6.9	981	19
14-Jul-04	11	$CdCl_2$	solvent control	28	2	8.82	7.45	936	19
14-Jul-04	11	$CdCl_2$	solvent control	28	3	8.72	7.5	934	19.1
14-Jul-04	11	$CdCl_2$	solvent control	28	4	8.68	7.17	1009	19.1
14-Jul-04	11	$CdCl_2$	solvent control	28	5	8.63	7.14	952	19.1
14-Jul-04	11	$CdCl_2$	0.02	28	1	8.64	6.87	947	19.1
14-Jul-04	11	$CdCl_2$	0.02	28	2	8.75	7.22	910	19.1
14-Jul-04	11	$CdCl_2$	0.02	28	3	8.62	6.79	982	19.1
14-Jul-04	11	$CdCl_2$	0.02	28	4	8.86	7.3	968	19.1
14-Jul-04	11	$CdCl_2$	0.02	28	5	8.74	7.41	932	19.1
14-Jul-04	11	$CdCl_2$	0.2	28	1	8.86	7.65	913	19
14-Jul-04	11	$CdCl_2$	0.2	28	2	8.77	7.39	935	19
14-Jul-04	11	$CdCl_2$	0.2	28	3	8.6	6.56	950	19
14-Jul-04	11	$CdCl_2$	0.2	28	4	8.68	6.89	983	19.1
14-Jul-04	11	$CdCl_2$	0.2	28	5	8.74	7.24	951	19
14-Jul-04	11	$CdCl_2$	2	28	1	8.74	7.39	922	18.9
14-Jul-04	11	$CdCl_2$	2	28	2	8.71	7.16	942	18.9
14-Jul-04	11	$CdCl_2$	2	28	3	8.81	7.44	959	19
14-Jul-04	11	$CdCl_2$	2	28	4	8.7	7.11	979	18.9
14-Jul-04	11	$CdCl_2$	2	28	5	8.63	6.42	1072	18.8
14-Jul-04	11	$CdCl_2$	20	28	1	8.57	6.51	1028	18.7
14-Jul-04	11	$CdCl_2$	20	28	2	8.65	6.76	980	18.8
14-Jul-04	11	$CdCl_2$	20	28	3	8.6	6.73	987	18.9
14-Jul-04	11	$CdCl_2$	20	28	4	8.7	7.24	943	18.9
14-Jul-04	11	$CdCl_2$	20	28	5	8.62	6.86	985	18.8
14-Jul-04	11	$CdCl_2$	200	28	1	8.61	7.28	943	18.8
14-Jul-04	11	$CdCl_2$	200	28	2	8.65	7.56	887	18.9
14-Jul-04	11	$CdCl_2$	200	28	3	8.6	7.5	886	18.9
14-Jul-04	11	$CdCl_2$	200	28	4	8.6	7.28	954	18.9
14-Jul-04	11	$CdCl_2$	200	28	5	8.64	7.39	917	18.7
12-Sep-03	7	DDT	control	0	1	8.2	6.85	927	20.2
12-Sep-03	7	DDT	control	0	2	8.14	6.68	918	20.1
12-Sep-03	7	DDT	control	0	3	8.24	7.13	910	20.1
12-Sep-03	7	DDT	control	0	4	8.06	5.73	950	20.1
12-Sep-03	7	DDT	control	0	5	8.34	6.65	945	20
12-Sep-03	7	DDT	solvent control	0	1	8.13	6.53	985	20.5
12-Sep-03	7	DDT	solvent control	0	2	8.18	6.71	962	20.4
12-Sep-03	7	DDT	solvent control	0	3	8.22	7.02	942	20.2
12-Sep-03	7	DDT	solvent control	0	4	8.22	7	989	20.3
12-Sep-03	7	DDT	solvent control	0	5	8.11	5.96	968	20.3
12-Sep-03	7	DDT	0.1	0	1	8.26	7.25	980	20.2
12-Sep-03	7	DDT	0.1	0	2	8.3	7.16	976	20.1
12-Sep-03	7	DDT	0.1	0	3	8.2	6.91	940	20.1
12-Sep-03	7	DDT	0.1	0	4	8.12	6.29	963	20.1
12-Sep-03	7	DDT	0.1	0	5	8.13	6.4	993	20.1
12-Sep-03	7	DDT	0.1	0	6	7.92	5.1	982	19.7
12-Sep-03	7	DDT	0.3	0	1	8.36	7.74	968	20
12-Sep-03	7	DDT	0.3	0	2	8.18	6.1	979	20
12-Sep-03	7	DDT	0.3	0	3	8.18	6.57	926	20
12-Sep-03	7	DDT	0.3	0	4	8.19	6.53	967	20.1
12-Sep-03	7	DDT	0.3	0	5	7.85	4.67	953	20.1
12-Sep-03	7	DDT	0.3	0	6	8.07	7.87	987	19.8
12-Sep-03	7	DDT	0.9	0	1	8.37	7.74	935	20.1
12-Sep-03	7	DDT	0.9	0	2	8.14	6.86	960	20
12-Sep-03	7	DDT	0.9	0	3	8.13	6.2	981	20
12-Sep-03	7	DDT	0.9	0	4	8.2	6.38	1015	20
12-Sep-03	7	DDT	0.9	0	5	8.25	7.17	961	19.9
12-Sep-03	7	DDT	0.9	0	6	8.18	7.72	977	19.8
12-Sep-03	7	DDT	2.7	0	1	8.14	7.07	927	20
12-Sep-03	7	DDT	2.7	0	2	8.16	6.33	964	19.8
12-Sep-03	7	DDT	2.7	0	3	8.19	6.86	994	20
12-Sep-03	7	DDT	2.7	0	4	8.12	5.93	957	20
12-Sep-03	7	DDT	2.7	0	5	8.17	6.83	936	19.9
12-Sep-03	7	DDT	2.7	0	6	8.13	8.18	936	19.8
12-Sep-03	7	DDT	8.1	0	1	8.31	7.26	947	20.1
12-Sep-03	7	DDT	8.1	0	2	8.38	7.65	924	20
12-Sep-03	7	DDT	8.1	0	3	8.32	7.02	958	19.9
12-Sep-03	7	DDT	8.1	0	4	8.27	6.91	909	19.9
12-Sep-03	7	DDT	8.1	0	5	8.16	6.23	950	19.9
12-Sep-03	7	DDT	8.1	0	6	8.22	9	943	19.8
10-Oct-03	7	DDT	control	28	1	8.32	7.6	889	19.5
10-Oct-03	7	DDT	control	28	2	8.41	7.83	922	19.5
10-Oct-03	7	DDT	control	28	3	8.53	7.68	880	19.4
10-Oct-03	7	DDT	control	28	4	8.57	7.74	886	19.4
10-Oct-03	7	DDT	control	28	5	8.51	7.65	888	19.4
10-Oct-03	7	DDT	solvent control	28	1	8.47	7.75	895	19.2
10-Oct-03	7	DDT	solvent control	28	2	8.6	7.62	924	19.2
10-Oct-03	7	DDT	solvent control	28	3	8.72	7.69	884	19.3
10-Oct-03	7	DDT	solvent control	28	4	8.64	7.67	917	19.3
10-Oct-03	7	DDT	solvent control	28	5	8.68	7.65	922	19.3

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Date	test number	chemical	concentration [mg kg^{-1}]	time [d]	rep	pH	O_2 [mg l^{-1}]	γ [μS cm^{-1}]	T [°C]
10-Oct-03	7	DDT	0.1	28	1	8.38	4.52	968	19
10-Oct-03	7	DDT	0.1	28	2	8.47	6.24	974	19.1
10-Oct-03	7	DDT	0.1	28	3	8.53	6.28	927	19.2
10-Oct-03	7	DDT	0.1	28	4	8.56	6.13	964	19.3
10-Oct-03	7	DDT	0.1	28	5	8.5	5.48	980	19.2
10-Oct-03	7	DDT	0.3	28	1	8.42	5.6	957	19.2
10-Oct-03	7	DDT	0.3	28	2	8.48	6.03	968	19.2
10-Oct-03	7	DDT	0.3	28	3	8.61	6.45	909	19.2
10-Oct-03	7	DDT	0.3	28	4	8.59	6.42	884	19.2
10-Oct-03	7	DDT	0.3	28	5	8.65	6.67	943	19.2
10-Oct-03	7	DDT	0.9	28	1	8.56	5.93	954	19.2
10-Oct-03	7	DDT	0.9	28	2	8.57	6.34	902	19.1
10-Oct-03	7	DDT	0.9	28	3	8.67	6.68	926	19.2
10-Oct-03	7	DDT	0.9	28	4	8.61	6.12	994	19.2
10-Oct-03	7	DDT	0.9	28	5	8.66	6.57	994	19.2
10-Oct-03	7	DDT	2.7	28	1	8.55	5.99	881	19.2
10-Oct-03	7	DDT	2.7	28	2	8.61	6.17	900	19.1
10-Oct-03	7	DDT	2.7	28	3	8.63	6.15	988	19.1
10-Oct-03	7	DDT	2.7	28	4	8.61	6.55	943	19.2
10-Oct-03	7	DDT	2.7	28	5	8.65	6.7	911	19.1
10-Oct-03	7	DDT	8.1	28	1	8.76	6.9	903	19.2
10-Oct-03	7	DDT	8.1	28	2	8.75	6.66	908	19.3
10-Oct-03	7	DDT	8.1	28	3	8.62	6.22	942	19.3
10-Oct-03	7	DDT	8.1	28	4	8.45	4.04	982	19.4
10-Oct-03	7	DDT	8.1	28	5	8.36	3.16	983	19.3
13-Feb-03	2	PCP	0.05	0	1	8.28	6.24		20.3
13-Feb-03	2	PCP	0.05	0	2	8.29	6.21	1010	20.2
13-Feb-03	2	PCP	0.05	0	3	8.35	6.76	1025	20.1
13-Feb-03	2	PCP	0.05	0	4	8.17	5.16	1036	20
13-Feb-03	2	PCP	0.05	0	5	8.41	6.41	1005	20.2
13-Feb-03	2	PCP	0.05	0	6	8.43	6.42	1032	20.2
13-Feb-03	2	PCP	c	0	1	8.45	6.85	1047	20.6
13-Feb-03	2	PCP	c	0	2	8.47	7.43	970	20.6
13-Feb-03	2	PCP	c	0	3	8.52	7.52	1000	20.5
13-Feb-03	2	PCP	c	0	4	8.5	6.95	1014	20.4
13-Feb-03	2	PCP	c	0	5	8.34	6.53	890	20.4
13-Feb-03	2	PCP	c	0	6	8.48	7.18	1047	20.7
13-Feb-03	2	PCP	sc	0	1	8.45	6.59	968	20.7
13-Feb-03	2	PCP	sc	0	2	8.35	6.84	952	20.6
13-Feb-03	2	PCP	sc	0	3	8.43	7.22	1061	20.5
13-Feb-03	2	PCP	sc	0	4	8.38	6.92	990	20.4
13-Feb-03	2	PCP	sc	0	5	8.48	7.33	1016	20.3
13-Feb-03	2	PCP	sc	0	6	8.02	7.52	954	20.3
13-Feb-03	2	PCP	0.5	0	1	8.3	5.71	1019	20.2
13-Feb-03	2	PCP	0.5	0	2	8.38	6.18	1035	20.2
13-Feb-03	2	PCP	0.5	0	3	8.16	4.45	1035	20.3
13-Feb-03	2	PCP	0.5	0	4	8.37	6.15	978	20.1
13-Feb-03	2	PCP	0.5	0	5	8.5	6.97	985	20.2
13-Feb-03	2	PCP	0.5	0	6	8.31	5.93	1028	20
13-Feb-03	2	PCP	5	0	1	8.42	7.04		20.4
13-Feb-03	2	PCP	5	0	2	8.47	7.23		20.4
13-Feb-03	2	PCP	5	0	3	8.43	7.15		20.4
13-Feb-03	2	PCP	5	0	4	8.38	6.4		20.4
13-Feb-03	2	PCP	5	0	5	8.34	6.14		20.5
13-Feb-03	2	PCP	5	0	6	8.42	6.65		20.4
13-Feb-03	2	PCP	50	0	1	8.36	6.93	963	20.2
13-Feb-03	2	PCP	50	0	2	8.1	5.98	1042	20.1
13-Feb-03	2	PCP	50	0	3	8.26	7.3	1012	20.1
13-Feb-03	2	PCP	50	0	4	8.1	7.04	972	20.3
13-Feb-03	2	PCP	50	0	5	8.04	7.11	984	20.1
13-Feb-03	2	PCP	50	0	6	8.03	6.8	982	20.1
13-Feb-03	2	PCP	500	0	1	8.2	7.97	840	20.2
13-Feb-03	2	PCP	500	0	2	8.15	7.26	893	20
13-Feb-03	2	PCP	500	0	3	8.11	6.68	930	19.7
13-Feb-03	2	PCP	500	0	4	8.42	7.32	889	20
13-Feb-03	2	PCP	500	0	5	8.27	5.54	927	20
13-Feb-03	2	PCP	500	0	6	8.4	7.13	843	19.9
13-Mar-03	2	PCP	0.05	28	1	8.56	7.47	907	20.5
13-Mar-03	2	PCP	0.05	28	2	8.71	8.1	878	20.3
13-Mar-03	2	PCP	0.05	28	3	8.72	8.12	1052	20.2
13-Mar-03	2	PCP	0.05	28	4	8.54	6.82	956	20.1
13-Mar-03	2	PCP	0.05	28	5	8.68	7.92	989	20.1
13-Mar-03	2	PCP	c	28	1	8.75	8.21	918	20.2
13-Mar-03	2	PCP	c	28	2	8.68	7.97	992	20.1
13-Mar-03	2	PCP	c	28	3	8.66	7.92	929	20.2
13-Mar-03	2	PCP	c	28	4	8.3	7.89	927	20.1
13-Mar-03	2	PCP	c	28	5	8.66	7.93	854	20
13-Mar-03	2	PCP	sc	28	1	8.53	7.31	906	20.4
13-Mar-03	2	PCP	sc	28	2	8.61	7.68	955	20.1
13-Mar-03	2	PCP	sc	28	3	8.72	8.05	948	20.1
13-Mar-03	2	PCP	sc	28	4	8.6	7.78	913	20.1
13-Mar-03	2	PCP	sc	28	5	8.52	7.3	980	20.1
13-Mar-03	2	PCP	0.5	28	1	8.67	7.96	953	20.1
13-Mar-03	2	PCP	0.5	28	2	8.35	7.05	955	20.3
13-Mar-03	2	PCP	0.5	28	3	8.76	8.16	885	20.3
13-Mar-03	2	PCP	0.5	28	4	8.52	7.02	943	20.2
13-Mar-03	2	PCP	0.5	28	5	8.66	7.7	966	20.2
13-Mar-03	2	PCP	5	28	1	8.56	7.63	944	20.4
13-Mar-03	2	PCP	5	28	2	8.59	7.67	903	20.3
13-Mar-03	2	PCP	5	28	3	8.6	7.67	972	20.3

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Date	test number	chemical	concentration [mg kg^{-1}]	time [d]	rep	pH	O_2 [mg l^{-1}]	γ [μS cm^{-1}]	T [°C]
13-Mar-03	2	PCP	5	28	4	8.48	5.97	1074	20.3
13-Mar-03	2	PCP	5	28	5	8.64	7.72	962	20.2
13-Mar-03	2	PCP	50	28	1		7.15	935	20.2
13-Mar-03	2	PCP	50	28	2		7.45	985	20.1
13-Mar-03	2	PCP	50	28	3		7.14	898	20.1
13-Mar-03	2	PCP	50	28	4		6.98	952	18.8
13-Mar-03	2	PCP	50	28	5		5.08	946	20.1
13-Mar-03	2	PCP	500	28	1		7.74	764	20
13-Mar-03	2	PCP	500	28	2		7.7	843	20
13-Mar-03	2	PCP	500	28	3		7.56	852	20.1
13-Mar-03	2	PCP	500	28	4		7.67	805	20
13-Mar-03	2	PCP	500	28	5		7.85	770	20.1
06-Feb-03	1	TBT-Cl	c	0	1	8.35	7.3	1004	19.3
06-Feb-03	1	TBT-Cl	c	0	2	8.29	7.16	932	19.3
06-Feb-03	1	TBT-Cl	c	0	3	8.17	5.64	982	19.5
06-Feb-03	1	TBT-Cl	c	0	4	8.35	7.04	1065	19.8
06-Feb-03	1	TBT-Cl	c	0	5	8.27	6.72	957	19.8
06-Feb-03	1	TBT-Cl	c	0	6	8.18	5.96	999	19.3
06-Feb-03	1	TBT-Cl	sc	0	1	8.03	7.35	1025	19.7
06-Feb-03	1	TBT-Cl	sc	0	2	8.16	7.05	990	19.8
06-Feb-03	1	TBT-Cl	sc	0	3	8.41	6.89	1069	20.2
06-Feb-03	1	TBT-Cl	sc	0	4	7.97	2.64	1073	19.3
06-Feb-03	1	TBT-Cl	sc	0	5	8.17	5.15	1044	19.8
06-Feb-03	1	TBT-Cl	sc	0	6	8.21	6.74	1011	19.8
06-Feb-03	1	TBT-Cl	1	0	1	8.17	6.12	1025	20.2
06-Feb-03	1	TBT-Cl	1	0	2	8.26	6.71	995	20.3
06-Feb-03	1	TBT-Cl	1	0	3	8.29	6.86	960	20.3
06-Feb-03	1	TBT-Cl	1	0	4	8.36	7.1	997	19.8
06-Feb-03	1	TBT-Cl	1	0	5	8.38	7.22	1085	19.8
06-Feb-03	1	TBT-Cl	1	0	6	8.31	6.5	1060	20
06-Feb-03	1	TBT-Cl	2	0	1	8.28	6.76	1020	19.5
06-Feb-03	1	TBT-Cl	2	0	2	8.37	6.96	996	19.8
06-Feb-03	1	TBT-Cl	2	0	3	8.36	7.42	1034	20.1
06-Feb-03	1	TBT-Cl	2	0	4	8.23	4.46	1012	20.2
06-Feb-03	1	TBT-Cl	2	0	5	8.27	5.66	1013	20.2
06-Feb-03	1	TBT-Cl	2	0	6	8.34	6.03	984	20.2
06-Feb-03	1	TBT-Cl	4	0	1	8.17	9.45	978	19.5
06-Feb-03	1	TBT-Cl	4	0	2	8.3	10.5	1033	19.9
06-Feb-03	1	TBT-Cl	4	0	3	8.19	10.7	974	20.1
06-Feb-03	1	TBT-Cl	4	0	4	8.18	9.81	959	20.2
06-Feb-03	1	TBT-Cl	4	0	5	8.4	8.37	1076	20.2
06-Feb-03	1	TBT-Cl	4	0	6	8.3	8.63	940	20.2
06-Feb-03	1	TBT-Cl	8	0	1	8.11	5.9	1138	20.4
06-Feb-03	1	TBT-Cl	8	0	2	8.18	5.54	1074	20.4
06-Feb-03	1	TBT-Cl	8	0	3	8.19	6.45	1089	20.4
06-Feb-03	1	TBT-Cl	8	0	4	8.37	5.9	1070	20.4
06-Feb-03	1	TBT-Cl	8	0	5	8.27	5.62	994	20.4
06-Feb-03	1	TBT-Cl	8	0	6	8.36	5.51	984	20.3
06-Feb-03	1	TBT-Cl	16	0	1	7.04	10.27	1020	20.3
06-Feb-03	1	TBT-Cl	16	0	2	8.1	7.96	986	20.3
06-Feb-03	1	TBT-Cl	16	0	3	7.48	9.63	931	20.3
06-Feb-03	1	TBT-Cl	16	0	4	7.3	8.04	977	20.2
06-Feb-03	1	TBT-Cl	16	0	5	7.74	7.08	909	20.1
06-Feb-03	1	TBT-Cl	16	0	6	8.27	8.79	1069	20.1
06-Mar-03	1	TBT-Cl	c	28	1	8.63	7.92	1006	19.7
06-Mar-03	1	TBT-Cl	c	28	2	8.7	7.62	975	19.9
06-Mar-03	1	TBT-Cl	c	28	3	8.56	7.28	966	20.2
06-Mar-03	1	TBT-Cl	c	28	4	8.47	7.47	974	19.7
06-Mar-03	1	TBT-Cl	c	28	5	8.28	5.42	978	20
06-Mar-03	1	TBT-Cl	sc	28	1	8.29	6.62	1048	19.4
06-Mar-03	1	TBT-Cl	sc	28	2	7.61	7.55	957	19.8
06-Mar-03	1	TBT-Cl	sc	28	3	8.51	8.22	948	19.8
06-Mar-03	1	TBT-Cl	sc	28	4	8.35	7.84	1010	19.7
06-Mar-03	1	TBT-Cl	sc	28	5	7.79	6.98	1008	19.9
06-Mar-03	1	TBT-Cl	1	28	1	8.45	6.4	1019	18.8
06-Mar-03	1	TBT-Cl	1	28	2	8.66	7.8	1036	18.4
06-Mar-03	1	TBT-Cl	1	28	3	8.59	7.54	962	19.4
06-Mar-03	1	TBT-Cl	1	28	4	8.4	7.48	937	19.8
06-Mar-03	1	TBT-Cl	1	28	5	8.68	8.01	1006	19.5
06-Mar-03	1	TBT-Cl	2	28	1	8.73	8.01	1018	18.6
06-Mar-03	1	TBT-Cl	2	28	2	8.57	7.62	1076	19.2
06-Mar-03	1	TBT-Cl	2	28	3	8.51	6.59	936	19.1
06-Mar-03	1	TBT-Cl	2	28	4	8.66	8.11	975	19
06-Mar-03	1	TBT-Cl	2	28	5	8.3	6.8	1008	18.9
06-Mar-03	1	TBT-Cl	4	28	1	8.62	7.72	962	19
06-Mar-03	1	TBT-Cl	4	28	1	8.41	6.44	991	18.9
06-Mar-03	1	TBT-Cl	4	28	2	8.5	6.68	1010	18.8
06-Mar-03	1	TBT-Cl	4	28	3	8.67	7.97	938	18.8
06-Mar-03	1	TBT-Cl	4	28	4	8.56	6.97	1090	18.7
06-Mar-03	1	TBT-Cl	4	28	5	8.42	6.48	1037	18.8
06-Mar-03	1	TBT-Cl	8	28	2	8.43	7.16	993	18.5
06-Mar-03	1	TBT-Cl	8	28	3	8.64	8.3	1051	18.4
06-Mar-03	1	TBT-Cl	8	28	4	8.56	7.76	1053	18.7
06-Mar-03	1	TBT-Cl	8	28	5	8.53	7.78	1024	19.5
06-Mar-03	1	TBT-Cl	16	28	1	8.03	3.68	930	19.9
06-Mar-03	1	TBT-Cl	16	28	2	8.28	6.46	994	19
06-Mar-03	1	TBT-Cl	16	28	3	8.36	7.3	921	18.9
06-Mar-03	1	TBT-Cl	16	28	4	8.28	6.51	991	18.9
06-Mar-03	1	TBT-Cl	16	28	5	8.31	5.68	980	18.7
28-Mar-03	3	TNT	control	0	1	8.21	7.36	979	19.5

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Date	test number	chemical	concentration [mg kg^{-1}]	time [d]	rep	pH	O_2 [mg l^{-1}]	γ [μS cm^{-1}]	T [°C]
28-Mar-03	3	TNT	control	0	2	8.41	8.17	993	19.5
28-Mar-03	3	TNT	control	0	3	8.15	9.11	959	19.2
28-Mar-03	3	TNT	control	0	4	8.09	6.44	978	20.4
28-Mar-03	3	TNT	control	0	5	8.3	7.72	1015	19.6
28-Mar-03	3	TNT	control	0	6	8.28	7.85	1004	19.2
28-Mar-03	3	TNT	solvent control	0	1	8.24	7.48	1017	19.7
28-Mar-03	3	TNT	solvent control	0	2	8.11	6.17	1005	19.7
28-Mar-03	3	TNT	solvent control	0	3	8.08	5.15	955	20.3
28-Mar-03	3	TNT	solvent control	0	4	8.24	7.61	1035	19.7
28-Mar-03	3	TNT	solvent control	0	5	8.35	7.94	967	19.5
28-Mar-03	3	TNT	solvent control	0	6	8.35	7.88	988	19.6
28-Mar-03	3	TNT	5.12	0	1	8.27	7.42	862	19.7
28-Mar-03	3	TNT	5.12	0	2	8.3	7.73	974	19.5
28-Mar-03	3	TNT	5.12	0	3	8.23	6.63	987	19.6
28-Mar-03	3	TNT	5.12	0	4	8.12	5.45	1008	20.2
28-Mar-03	3	TNT	5.12	0	5	8.29	7.1	997	20
28-Mar-03	3	TNT	5.12	0	6	8.4	7.66	1046	20
28-Mar-03	3	TNT	12.8	0	1	8.32	7.59	956	19.9
28-Mar-03	3	TNT	12.8	0	2	8.13	5.95	1002	19.8
28-Mar-03	3	TNT	12.8	0	3	8.02	5.23	1023	19.8
28-Mar-03	3	TNT	12.8	0	4	8.31	7.87	974	20.3
28-Mar-03	3	TNT	12.8	0	5	8.23	7.29	1007	19.7
28-Mar-03	3	TNT	12.8	0	6	8.35	7.77	1026	19.6
28-Mar-03	3	TNT	32	0	1	8.32	7.3	1006	20
28-Mar-03	3	TNT	32	0	2	8.32	7.66	989	19.9
28-Mar-03	3	TNT	32	0	3	8.41	8.19	945	19.8
28-Mar-03	3	TNT	32	0	4	8.24	7.12	972	19.9
28-Mar-03	3	TNT	32	0	5	8.33	7.81	956	20
28-Mar-03	3	TNT	32	0	6	8.22	6.79	1003	20.4
28-Mar-03	3	TNT	80	0	1	8.22	7.13	968	19.5
28-Mar-03	3	TNT	80	0	2	8.33	7.41	946	19.8
28-Mar-03	3	TNT	80	0	3	8.24	6.84	992	19.9
28-Mar-03	3	TNT	80	0	4	8.29	7.36	975	19.8
28-Mar-03	3	TNT	80	0	5	8.3	8.19	964	20.4
28-Mar-03	3	TNT	80	0	6	8.29	7.55	978	19.8
28-Mar-03	3	TNT	200	0	1	8.31	8.03	942	20.3
28-Mar-03	3	TNT	200	0	2	8.29	7.63	949	19.6
28-Mar-03	3	TNT	200	0	3	8.31	7.92	954	19.5
28-Mar-03	3	TNT	200	0	4	8.3	7.47	946	19.7
28-Mar-03	3	TNT	200	0	5	8.36	7.58	1005	19.8
28-Mar-03	3	TNT	200	0	6	8.36	7.85	962	19.8
24-Apr-03	3	TNT	control	28	1	8.58	7.94	968	20.4
24-Apr-03	3	TNT	control	28	2	8.5	7.26	953	20.3
24-Apr-03	3	TNT	control	28	3	8.4	5.81	944	20.4
24-Apr-03	3	TNT	control	28	5	8.53	7.49	943	20.3
24-Apr-03	3	TNT	control	28	6	8.54	7.48	911	20.3
24-Apr-03	3	TNT	solvent control	28	1	8.53	6.96	969	20.1
24-Apr-03	3	TNT	solvent control	28	2	8.48	7.08	950	20.3
24-Apr-03	3	TNT	solvent control	28	4	8.53	7.13	902	20.4
24-Apr-03	3	TNT	solvent control	28	5	8.59	7.58	919	20.4
24-Apr-03	3	TNT	solvent control	28	6	8.64	8.01	883	20.4
24-Apr-03	3	TNT	5.12	28	1	8.47	7.41	936	20.6
24-Apr-03	3	TNT	5.12	28	2	8.64	8.26	880	20.6
24-Apr-03	3	TNT	5.12	28	3	8.65	7.92	878	20.5
24-Apr-03	3	TNT	5.12	28	5	8.44	6.86	1009	20.5
24-Apr-03	3	TNT	5.12	28	6	8.45	7.01	1013	20.5
24-Apr-03	3	TNT	12.8	28	1	8.53	7.45	947	20.6
24-Apr-03	3	TNT	12.8	28	2	8.46	7	885	20.6
24-Apr-03	3	TNT	12.8	28	3	8.47	6.88	943	20.6
24-Apr-03	3	TNT	12.8	28	5	8.45	6.67	975	20.6
24-Apr-03	3	TNT	12.8	28	6	8.42	7.24	926	20.6
24-Apr-03	3	TNT	32	28	1	8.47	7.43	931	20.7
24-Apr-03	3	TNT	32	28	2	8.34	6.41	908	20.6
24-Apr-03	3	TNT	32	28	3	8.38	6.82	977	20.6
24-Apr-03	3	TNT	32	28	4	8.43	6.97	950	20.5
24-Apr-03	3	TNT	32	28	6	8.45	7.28	946	20.6
24-Apr-03	3	TNT	80	28	1	8.5	6.98	993	20.3
24-Apr-03	3	TNT	80	28	2	8.44	7.06	931	20.4
24-Apr-03	3	TNT	80	28	3	8.47	5.9	908	20.4
24-Apr-03	3	TNT	80	28	4	8.57	7.8	920	20.5
24-Apr-03	3	TNT	80	28	6	8.47	7.23	961	20.6
24-Apr-03	3	TNT	200	28	2	8.5	7.71	930	20.7
24-Apr-03	3	TNT	200	28	3	8.47	6.86	924	20.7
24-Apr-03	3	TNT	200	28	4	8.52	7.27	957	20.7
24-Apr-03	3	TNT	200	28	5	8.52	7.37	948	20.7
24-Apr-03	3	TNT	200	28	6	8.37	6.25	995	20.7

Table 7.5: Effect data of 48-hour acute toxicity tests with *C. riparius*

chemical	nominal concentration	unit	number exposed number exposed	number with lethal effects	observed proportion with lethal effects [%]
$CdCl_2$	control		10	0	0
$CdCl_2$	control (tapwater)		10	0	0
$CdCl_2$	3.125	mg l^{-1}	10	0	0
$CdCl_2$	6.5	mg l^{-1}	10	4	40
$CdCl_2$	12.5	mg l^{-1}	10	8	80
$CdCl_2$	25	mg l^{-1}	10	10	100
$CdCl_2$	50	mg l^{-1}	10	10	100
$CdCl_2$	100	mg l^{-1}	10	10	100
3,4-DCA	control		11	0	0
3,4-DCA	2.5	mg l^{-1}	11	1	9
3,4-DCA	5	mg l^{-1}	9	1	11
3,4-DCA	10	mg l^{-1}	11	3	27
3,4-DCA	20	mg l^{-1}	10	10	100
3,4-DCA	40	mg l^{-1}	10	10	100

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chemical	nominal concentration	unit	number exposed number exposed	number with lethal effects	observed proportion with lethal effects [%]
PCP	control		12	0	0
PCP	solvent control		11	0	0
PCP	0.25	$mg l^{-1}$	12	0	0
PCP	0.5	$mg l^{-1}$	11	0	0
PCP	1	$mg l^{-1}$	11	1	9
PCP	2	$mg l^{-1}$	11	9	82
PCP	4	$mg l^{-1}$	12	12	100
2,4-DCP	control		12	0	0
2,4-DCP	1.333	$mg l^{-1}$	11	0	0
2,4-DCP	2	$mg l^{-1}$	11	0	0
2,4-DCP	3	$mg l^{-1}$	12	0	0
2,4-DCP	4.5	$mg l^{-1}$	12	0	0
2,4-DCP	6.75	$mg l^{-1}$	12	4	33
2,4-DCP	10.125	$mg l^{-1}$	12	12	100
TBT-Cl	control		11	0	0
TBT-Cl	solvent control		11	0	0
TBT-Cl	45.88	$\mu g l^{-1}$	12	0	0
TBT-Cl	61.17	$\mu g l^{-1}$	10	0	0
TBT-Cl	81.56	$\mu g l^{-1}$	12	1	8
TBT-Cl	108.75	$\mu g l^{-1}$	10	4	40
TBT-Cl	145	$\mu g l^{-1}$	11	6	55
TNT	control		12	0	0
TNT	solvent control		12	0	0
TNT	7.07	$mg l^{-1}$	9	0	0
TNT	10	$mg l^{-1}$	14	0	0
TNT	14.14	$mg l^{-1}$	12	7	58
TNT	20	$mg l^{-1}$	13	13	100
TNT	28.28	$mg l^{-1}$	12	12	100
TNT	40	$mg l^{-1}$	14	14	100
DDT	control		12	0	0
DDT	solvent control		12	0	0
DDT	0.0028	$mg l^{-1}$	12	0	0
DDT	0.0051	$mg l^{-1}$	11	2	18
DDT	0.0092	$mg l^{-1}$	10	3	30
DDT	0.0165	$mg l^{-1}$	11	3	27
DDT	0.0297	$mg l^{-1}$	9	7	78
DDT	0.0535	$mg l^{-1}$	9	9	100
B(a)P	up to 2	$mg l^{-1}$			0

Table 7.6: Effect data of 96-hour acute toxicity tests with *L. variegatus*

chemical	nominal concentration	unit	number exposed number exposed	number with lethal effects	observed proportion with lethal effects [%]
$CdCl_2$	control		10	0	0
$CdCl_2$	0.206	$mg l^{-1}$	10	0	0
$CdCl_2$	0.291	$mg l^{-1}$	10	0	0
$CdCl_2$	0.412	$mg l^{-1}$	10	1	10
$CdCl_2$	0.582	$mg l^{-1}$	10	8	80
$CdCl_2$	0.823	$mg l^{-1}$	10	10	100
$CdCl_2$	1.164	$mg l^{-1}$	10	10	100
3,4-DCA	control		9	0	0
3,4-DCA	2.5	$mg l^{-1}$	10	0	0
3,4-DCA	5	$mg l^{-1}$	10	0	0
3,4-DCA	10	$mg l^{-1}$	10	2	20
3,4-DCA	20	$mg l^{-1}$	10	10	100
3,4-DCA	40	$mg l^{-1}$	10	10	100
PCP	control		12	0	0
PCP	solvent control		12	0	0
PCP	0.31	$mg l^{-1}$	12	2	17
PCP	0.42	$mg l^{-1}$	12	12	100
PCP	0.56	$mg l^{-1}$	12	12	100
PCP	0.74	$mg l^{-1}$	12	12	100
PCP	0.99	$mg l^{-1}$	12	12	100
PCP	1.98	$mg l^{-1}$	12	12	100
2,4-DCP	control		10	0	0
2,4-DCP	8.84	$mg l^{-1}$	10	0	0
2,4-DCP	12.5	$mg l^{-1}$	10	0	0
2,4-DCP	17.68	$mg l^{-1}$	10	1	10
2,4-DCP	25	$mg l^{-1}$	10	8	80
2,4-DCP	35.36	$mg l^{-1}$	10	10	100
2,4-DCP	50	$mg l^{-1}$	10	10	100
TBT-Cl	control		10	0	0
TBT-Cl	solvent control		10	0	0
TBT-Cl	5	$\mu g l^{-1}$	10	0	0
TBT-Cl	7.1	$\mu g l^{-1}$	10	0	0
TBT-Cl	10	$\mu g l^{-1}$	10	2	20
TBT-Cl	1.41	$\mu g l^{-1}$	10	9	90
TBT-Cl	2	$\mu g l^{-1}$	10	10	100
TNT	control		10	0	0
TNT	7.07	$mg l^{-1}$	8	1	13
TNT	10	$mg l^{-1}$	9	7	78
TNT	14.140	$mg l^{-1}$	8	7	88
TNT	20	$mg l^{-1}$	8	8	100
TNT	28.280	$mg l^{-1}$	8	8	100

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chemical	nominal concentration	unit	number exposed number exposed	number with lethal effects	observed proportion with lethal effects [%]
TNT	40	$mg l^{-1}$	8	8	100
DDT	control		10	0	0
DDT	solvent control		10	0	0
DDT	0.28	$mg l^{-1}$	10	3	30
DDT	0.56	$mg l^{-1}$	10	3	30
DDT	1.125	$mg l^{-1}$	10	5	50
DDT	2.25	$mg l^{-1}$	10	10	100
DDT	4.5	$mg l^{-1}$	10	10	100
B(a)P	up to 2	$mg l^{-1}$			0

Table 7.7: Raw data of observed endpoints of the 28-day sediment toxicity tests with *L. variegatus*, 10 worms were exposed at start of the tests

chemical	concentration [$mg kg^{-1}$]	replicate	total number of surviving worms	worm dry weight [mg]	indiv. dry weight [mg]
2,4-DCP	c	1	34	34.66	1.02
2,4-DCP	sc	1	37	32.69	0.88
2,4-DCP	1.6	1	34	33.9	1.00
2,4-DCP	8	1	35	43.99	1.26
2,4-DCP	40	1	31	32.54	1.05
2,4-DCP	200	1	9	3.75	0.42
2,4-DCP	1000	1	0	0	0.00
B(a)p	c	1	58	50.3	0.87
B(a)p	sc	1	60	54.29	0.90
B(a)p	0.064	1	66	57.86	0.88
B(a)p	0.32	1	65	43.45	0.67
B(a)p	1.6	1	58	48.19	0.83
B(a)p	8	1	60	54.16	0.90
B(a)p	40	1	42	29.21	0.70
B(a)p	200	1	43	36.59	0.85
B(a)p	1000	1	31	13.63	0.44
$CdCl_2$	c	1	45	22.15	0.49
$CdCl_2$	sc	1	77	45.78	0.59
$CdCl_2$	0.0128	1	44	30.59	0.70
$CdCl_2$	0.064	1	73	40.62	0.56
$CdCl_2$	0.32	1	50	36.98	0.74
$CdCl_2$	1.6	1	40	25.94	0.65
$CdCl_2$	8	1	42	35.61	0.85
$CdCl_2$	40	1	10	8.91	0.89
$CdCl_2$	200	1	0	0	
DDT	c	1	32	29.01	0.91
DDT	c	2	44	35.45	0.81
DDT	c	3	69	52.75	0.76
DDT	c	4	40	22.62	0.57
DDT	c	5	25	22.79	0.91
DDT	sc	1	37	22.89	0.62
DDT	sc	2	23	16.27	0.71
DDT	sc	3	31	22.12	0.71
DDT	sc	4	28	17.34	0.62
DDT	sc	5	22	15	0.68
DDT	sc	6	11	8.09	0.74
DDT	0.2	1	59	49.13	0.83
DDT	0.2	2	34	30.97	0.91
DDT	0.2	3	34	26.77	0.79
DDT	0.2	4	32	30.55	0.95
DDT	0.2	5	25	16.8	0.67
DDT	1.41	1	26	20.18	0.78
DDT	1.41	2	32	21.85	0.68
DDT	1.41	3	49	53.36	1.09
DDT	1.41	4	57	54.39	0.95
DDT	1.41	5	45	31.17	0.69
DDT	10	1	24	14.49	0.60
DDT	10	2	22	14.33	0.65
DDT	10	3	29	30.56	1.05
DDT	10	4	30	25.94	0.86
DDT	70.71	1	23	35.15	1.53
DDT	70.71	2	10	5.96	0.60
DDT	70.71	3	11	11.59	1.05
DDT	70.71	4	24	33.32	1.39
DDT	70.71	5	10	8.02	0.80
DDT	500	1	6	1.05	0.17
DDT	500	2	0	0	
DDT	500	3	10	3.64	0.36
DDT	500	4	6	2.26	0.38
DDT	500	5	8	4.11	0.51
PCP	c	1	34	34.66	1.02
PCP	sc	1	37	32.69	0.88
PCP	0.064	1	38	39.28	1.03
PCP	0.32	1	48	58.64	1.22
PCP	1.6	1	51	44.23	0.87
PCP	8	1	41	31.03	0.76
PCP	40	1	0	0	
PCP	200	1	0	0	
PCP	1000	1	0	0	

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chemical	concentration [mg kg^{-1}]	replicate	total number of surviving worms	worm dry weight [mg]	indiv. dry weight [mg]
PCP *	control	1	26	14.11	0.54
PCP *	control	2	29	24.44	0.84
PCP *	control	3	39	28.46	0.73
PCP *	control	4	27	25.47	0.94
PCP *	control	5	35	23.48	0.67
PCP *	control	6	15	21.97	1.46
PCP *	solvent control	1	30	22.72	0.76
PCP *	solvent control	2	32	20.79	0.65
PCP *	solvent control	3	30	30.42	1.01
PCP *	0.05	1	28	21.56	0.77
PCP *	0.05	2	38	29.08	0.77
PCP *	0.05	3	20	15.06	0.75
PCP *	0.250	1	23	17.21	0.75
PCP *	0.250	2	21	23.53	1.12
PCP *	0.250	3	29	23.09	0.80
PCP *	1.250	1	22	16.72	0.76
PCP *	1.250	2	34	26.9	0.79
PCP *	1.250	3	19	13.76	0.72
PCP *	6.250	1	29	19.75	0.68
PCP *	6.250	2	27	17.51	0.65
PCP *	6.250	3	31	20.08	0.65
PCP *	31.250	1	0	0	
PCP *	31.250	2	0	0	
PCP *	31.250	3	0	0	
TBT-Cl	c	1	33	18.23	0.55
TBT-Cl	sc	1	23	15.76	0.69
TBT-Cl	0.0064	1	47	27.22	0.58
TBT-Cl	0.032	1	51	34.97	0.69
TBT-Cl	0.16	1	38	24.81	0.65
TBT-Cl	0.8	1	28	19.81	0.71
TBT-Cl	4	1	5	1.59	0.32
TBT-Cl	20	1	0	0	
TBT-Cl	100	1	0	0	
TNT	c	1	43	28.62	0.67
TNT	sc	1	46	33.38	0.73
TNT	0.032	1	36	35.01	0.97
TNT	0.16	1	55	35.6	0.65
TNT	0.8	1	50	41.94	0.84
TNT	4	1	45	67.14	1.49
TNT	20	1	44	37.95	0.86
TNT	100	1	33	36.77	1.11
TNT	500	1	0	0	

* = test was performed within the international ring test research and development project of the German Federal Environmental Agency

Table 7.8: Raw data of the 28-day sediment toxicity tests with *C. riparius*, 20 L1 larvae were exposed at the start of the test, E = emergence, i. dw = mean individual dry weight, dr = development rate, EMT_{50} = time at which 50% of the midges emerged

	conc.			number of emerging midges at day															i. dw	dr	EMT_{50}
chemical	[mg kg^{-1}]	rep.	sex	14	15	16	17	18	19	20	21	22	23	24	25	26	27	E	[mg]	[d^{-1}]	[d]
2,4-DCP	sc	1	m						2					1				3	0.83	0.050	19.91
2,4-DCP	sc	1	w						5	3	1							9	1.34	0.053	19.03
2,4-DCP	sc	2	m							n.E.								0			
2,4-DCP	sc	2	w					1	2	1								4	1.18	0.054	18.47
2,4-DCP	sc	3	m						1								1	2	0.70	0.046	21.79
2,4-DCP	sc	3	w					4	1	3						1		9	1.75	0.053	18.92
2,4-DCP	sc	4	m									1						1	1.25	0.047	21.50
2,4-DCP	sc	4	w							n.E.								0			
2,4-DCP	sc	5	m				1		2	1		1						5	0.71	0.053	18.76
2,4-DCP	sc	5	w					1	1	2	5	1	1					11	1.69	0.050	20.05
2,4-DCP	control	1	m					2		2	2	1	1					8	0.62	0.051	19.74
2,4-DCP	control	1	w					2		2	2	1	2					9	1.44	0.050	20.01
2,4-DCP	control	2	m			5	4		1									10	0.70	0.062	16.15
2,4-DCP	control	2	w				4		6									10	1.54	0.057	17.64
2,4-DCP	control	3	m							n.E.								0			
2,4-DCP	control	3	w							n.E.								0			
2,4-DCP	control	4	m			5	2	1										8	0.75	0.063	15.97
2,4-DCP	control	4	w				3		4									7	1.47	0.057	17.59
2,4-DCP	control	5	m		8	3												11	0.71	0.068	14.76
2,4-DCP	control	5	w			3	4	2										9	1.45	0.061	16.36
2,4-DCP	8	1	m						2			2						4	1.03	0.050	19.89
2,4-DCP	8	1	w					1		1	1					1		4	1.61	0.049	20.36
2,4-DCP	8	2	m				1		2			1						4	0.51	0.054	18.59
2,4-DCP	8	2	w		1	1	2	1		1		1						7	1.43	0.059	17.09
2,4-DCP	8	3	m				5	1										6	0.83	0.060	16.66
2,4-DCP	8	3	w				1	1	2									4	1.52	0.056	17.71
2,4-DCP	8	4	m					1				1						2	0.82	0.052	19.29
2,4-DCP	8	4	w						1		3		1					5	1.43	0.049	20.42
2,4-DCP	8	5	m					2			2						1	5	0.89	0.050	20.03
2,4-DCP	8	5	w								1	1						2	1.76	0.048	20.99
2,4-DCP	17.89	1	m							n.E.								0			
2,4-DCP	17.89	1	w							2	2							4	1.74	0.050	19.99
2,4-DCP	17.89	2	m		4	1	1		1									7	0.66	0.065	15.38
2,4-DCP	17.89	2	w			3	2	5	1	1								12	1.63	0.059	17.00
2,4-DCP	17.89	3	m			2												2	0.93	0.065	15.50
2,4-DCP	17.89	3	w			2	4	1										7	1.35	0.061	16.33
2,4-DCP	17.89	4	m					1	1							1		3	0.77	0.050	19.95
2,4-DCP	17.89	4	w						3		1							4	1.64	0.053	18.96
2,4-DCP	17.89	5	m			1	4											5	0.57	0.061	16.29
2,4-DCP	17.89	5	w		1		4	6										11	1.42	0.059	16.81
2,4-DCP	40	1-5								n.E.								0			
2,4-DCP	89	1-5								n.E.								0			
2,4-DCP	200	1-5								n.E.								0			
B(a)p	sc	1	m	6	6													12	0.66	0.072	13.98
B(a)p	sc	1	w		2	4												6	1.51	0.066	15.15
B(a)p	sc	2	m	5	5	1												11	0.64	0.071	14.11
B(a)p	sc	2	w		1		1											2	1.42	0.065	15.44
B(a)p	sc	3	m	1	6													7	0.67	0.070	14.35
B(a)p	sc	3	w		1	4	1											6	1.49	0.065	15.48
B(a)p	sc	4	m		4	4	1											9	0.66	0.066	15.14
B(a)p	sc	4	w			2	6	2										10	1.53	0.061	16.48
B(a)p	sc	5	m		3	3												6	0.66	0.067	14.98
B(a)p	sc	5	w			2	7	2										11	1.47	0.061	16.48
B(a)p	sc	6	m	2	5	4												11	0.69	0.068	14.65
B(a)p	sc	6	w			2	5	1										8	1.60	0.061	16.35
B(a)p	control	1	m	4	8													12	0.65	0.071	14.15
B(a)p	control	1	w		1	7												8	1.35	0.065	15.37
B(a)p	control	2	m		1	4	1											6	0.66	0.065	15.48
B(a)p	control	2	w			4	4	1										9	1.51	0.062	16.14
B(a)p	control	3	m	4	4	1												9	0.72	0.071	14.14
B(a)p	control	3	w			5	3											8	1.43	0.063	15.86
B(a)p	control	4	m			7	1	3										11	0.74	0.062	16.09
B(a)p	control	4	w				3	1		1								5	1.57	0.058	17.23
B(a)p	control	5	m		2	7	1	1										11	0.80	0.064	15.55
B(a)p	control	5	w				5	3	1									9	1.54	0.059	17.03
B(a)p	1000	1	m	3	8	5	1											17	0.69	0.068	14.69
B(a)p	1000	1	w			2												2	1.39	0.065	15.50
B(a)p	1000	2	m		3	4	1			1								9	0.66	0.064	15.60
B(a)p	1000	2	w			2	1			2								5	1.51	0.058	17.11
B(a)p	1000	3	m			1												1	0.58	0.065	15.50
B(a)p	1000	3	w			7	2	1										10	1.48	0.063	15.87
B(a)p	1000	4	m		1	6	1											8	0.69	0.065	15.48
B(a)p	1000	4	w				5	1										6	1.51	0.060	16.66
B(a)p	1000	5	m			5	1											6	0.71	0.064	15.66
B(a)p	1000	5	w			1	4	4	2									11	1.59	0.059	17.09
B(a)p	100	1	m		4	1	1	1										7	0.73	0.065	15.28
B(a)p	100	1	w			2	4	3		3								12	1.69	0.058	17.22
B(a)p	100	2	m		4	3	4		1									12	0.70	0.064	15.67
B(a)p	100	2	w			2	3		2	1								8	1.50	0.059	17.01
B(a)p	100	3	m	1	6													7	0.69	0.070	14.35

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	conc.			number of emerging midges at day															i. dw	dr	EMT_{50}
chemical	[mg kg^{-1}]	rep.	sex	14	15	16	17	18	19	20	21	22	23	24	25	26	27	E	[mg]	[d^{-1}]	[d]
B(a)p	100	3	w			5	1											6	1.57	0.064	15.66
B(a)p	100	4	m		2	5												7	0.65	0.066	15.20
B(a)p	100	4	w			6	2	3										11	1.55	0.062	16.18
B(a)p	100	5	m		2	5	3			1								11	0.77	0.063	15.86
B(a)p	100	5	w			5	3			1								9	1.67	0.062	16.20
B(a)p	10	1	m		8	1	1		1									11	0.77	0.066	15.05
B(a)p	10	1	w			2	2				1							5	1.48	0.060	16.72
B(a)p	10	2	m			5	2											7	0.70	0.063	15.77
B(a)p	10	2	w				3	2	3	1								9	1.53	0.057	17.66
B(a)p	10	3	m		3	2	1	2										8	0.65	0.064	15.66
B(a)p	10	3	w			2	3	2		1								8	1.58	0.060	16.79
B(a)p	10	4	m		1	6	1											8	0.71	0.065	15.48
B(a)p	10	4	w			3	2											5	1.54	0.063	15.89
B(a)p	10	5	m	2	6	2		1										11	0.68	0.068	14.70
B(a)p	10	5	w			5	1											6	1.49	0.064	15.66
B(a)p	1	1	m	3	5	1												9	0.68	0.070	14.25
B(a)p	1	1	w			6	1											7	1.42	0.064	15.64
B(a)p	1	2	m		3	5	1											9	0.71	0.066	15.25
B(a)p	1	2	w			4	3		2									9	1.50	0.061	16.42
B(a)p	1	3	m			2	5											7	0.71	0.062	16.20
B(a)p	1	3	w			1	7	2										10	1.57	0.060	16.58
B(a)p	1	4	m	1	5	4												10	0.66	0.068	14.77
B(a)p	1	4	w			6	1											7	1.54	0.064	15.64
B(a)p	1	5	m	1	6	2		1										10	0.67	0.067	14.84
B(a)p	1	5	w			5	2		1									8	1.48	0.062	16.07
B(a)p	0.1	1	m	5	4	2												11	0.65	0.070	14.19
B(a)p	0.1	1	w			6		1										7	1.58	0.063	15.76
B(a)p	0.1	2	m	1	4	3												8	0.68	0.068	14.72
B(a)p	0.1	2	w		1	7	2	2										12	1.47	0.063	15.87
B(a)p	0.1	3	m	1	4	1			1									7	0.68	0.067	14.94
B(a)p	0.1	3	w		3	5												8	1.51	0.066	15.11
B(a)p	0.1	4	m		6	1												7	0.66	0.068	14.63
B(a)p	0.1	4	w			5	3											8	1.40	0.063	15.86
B(a)p	0.1	5	m		2	4	4											10	0.69	0.064	15.66
B(a)p	0.1	5	w			2	2		1									5	1.49	0.061	16.43
$CdCl_2$	sc	1	m			11	3											14	0.73	0.064	15.70
$CdCl_2$	sc	1	w				2	1	2		1							6	1.66	0.056	17.90
$CdCl_2$	sc	2	m			7	4	1										12	0.73	0.063	15.97
$CdCl_2$	sc	2	w				2	4										6	1.54	0.058	17.15
$CdCl_2$	sc	3	m			4	3	1			1							9	0.70	0.061	16.49
$CdCl_2$	sc	3	w				5	5										10	1.52	0.059	16.99
$CdCl_2$	sc	4	m			5	1											6	0.75	0.064	15.66
$CdCl_2$	sc	4	w				7	4			1	1						13	1.56	0.058	17.38
$CdCl_2$	sc	5	m			1	3	5	1									10	0.71	0.059	17.06
$CdCl_2$	sc	5	w				3	5			1							9	1.56	0.057	17.43
$CdCl_2$	control	1	m			4	3											7	0.74	0.063	15.91
$CdCl_2$	control	1	w				7											7	1.57	0.061	16.50
$CdCl_2$	control	2	m			1	1			1								3	0.89	0.059	17.01
$CdCl_2$	control	2	w				2	1	2		1							6	1.63	0.056	17.90
$CdCl_2$	control	3	m			5	2	1										8	0.72	0.063	15.97
$CdCl_2$	control	3	w			3	8											11	1.59	0.062	16.21
$CdCl_2$	control	4	m		1	3												4	0.75	0.066	15.24
$CdCl_2$	control	4	w				2		1									3	1.83	0.058	17.12
$CdCl_2$	control	5	m		2	7	2	2	1									14	0.74	0.063	15.93
$CdCl_2$	control	5	w			3	1											4	1.61	0.064	15.74
$CdCl_2$	200	1-5								n.E.								0			
$CdCl_2$	20	1	m							n.E.								0			
$CdCl_2$	20	1	w									1						1	1.74	0.047	21.50
$CdCl_2$	20	2	m					1	1	1								3	0.67	0.054	18.46
$CdCl_2$	20	2	w					1		2								3	1.43	0.053	18.78
$CdCl_2$	20	3	m							1								1	0.72	0.051	19.50
$CdCl_2$	20	3	w							n.E.								0			
$CdCl_2$	20	4	m					1										1	0.72	0.057	17.50
$CdCl_2$	20	4	w							n.E.								0			
$CdCl_2$	20	5	m									1						1	0.79	0.047	21.50
$CdCl_2$	20	5	w							n.E.								0			
$CdCl_2$	2	1	m			9		2										11	0.69	0.063	15.83
$CdCl_2$	2	1	w				4	2	1	1								8	1.58	0.058	17.31
$CdCl_2$	2	2	m			5	4	2										11	0.69	0.062	16.19
$CdCl_2$	2	2	w			1	1	3		2								7	1.53	0.057	17.54
$CdCl_2$	2	3	m			8	4											12	0.68	0.063	15.82
$CdCl_2$	2	3	w				7	1										8	1.52	0.060	16.62
$CdCl_2$	2	4	m				2	1	2	4	1	2						12	0.64	0.053	18.95
$CdCl_2$	2	4	w					1		1	1	1			1			5	1.43	0.049	20.45
$CdCl_2$	2	5	m				9	2										11	0.73	0.060	16.67
$CdCl_2$	2	5	w				2	4	1	1								8	1.64	0.057	17.58
$CdCl_2$	0.2	1	m				2		1		1							4	0.80	0.056	17.85
$CdCl_2$	0.2	1	w				2	1	3	1					1			8	1.55	0.054	18.49
$CdCl_2$	0.2	2	m			1			1	1	1		1		2	1		8	0.68	0.048	20.84
$CdCl_2$	0.2	2	w					2				1			1			4	1.60	0.050	19.84
$CdCl_2$	0.2	3	m				2	2			1	1						6	0.69	0.055	18.14
$CdCl_2$	0.2	3	w				2	3		2	1							8	1.41	0.055	18.02
$CdCl_2$	0.2	4	m			4	5	1										10	0.71	0.062	16.18
$CdCl_2$	0.2	4	w			1	2	6	1									10	1.53	0.058	17.16
$CdCl_2$	0.2	5	m				1	1										2	0.67	0.059	16.99
$CdCl_2$	0.2	5	w						2	1								3	1.44	0.053	18.82
$CdCl_2$	0.02	1	m			4	3	2										9	0.62	0.062	16.24
$CdCl_2$	0.02	1	w				8	2		1	1							12	1.52	0.058	17.16
$CdCl_2$	0.02	2	m			7	1	1										9	0.76	0.063	15.81
$CdCl_2$	0.02	2	w				4	3		2								9	1.51	0.057	17.43

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	conc.			number of emerging midges at day															i. dw	dr	EMT_{50}
chemical	[mg kg^{-1}]	rep.	sex	14	15	16	17	18	19	20	21	22	23	24	25	26	27	E	[mg]	[d^{-1}]	[d]
$CdCl_2$	0.02	3	m			4	1			1								6	0.76	0.062	16.22
$CdCl_2$	0.02	3	w				5	4	1		1							11	1.56	0.058	17.34
$CdCl_2$	0.02	4	m			7	5	2										14	0.68	0.062	16.11
$CdCl_2$	0.02	4	w				2	2	1									5	1.65	0.058	17.27
$CdCl_2$	0.02	5	m			6	1											7	0.77	0.064	15.64
$CdCl_2$	0.02	5	w				5	6			1							12	1.56	0.058	17.27
DDT	sc	1	m			1	5	1										7	0.58	0.061	16.48
DDT	sc	1	w				3	4	3	2	1							13	1.44	0.056	17.96
DDT	sc	2	m				4	4	3		1							12	0.63	0.057	17.60
DDT	sc	2	w					1	4	1	1	1						8	1.49	0.052	19.05
DDT	sc	3	m				3	3	3									9	0.65	0.057	17.46
DDT	sc	3	w				1	1	5	4								11	1.49	0.054	18.55
DDT	sc	4	m			1	1	1		2	1	3			2			11	0.66	0.050	19.83
DDT	sc	4	w				1			1			1	2	1			6	1.48	0.047	21.26
DDT	sc	5	m						3	5	3	1						12	0.71	0.051	19.63
DDT	sc	5	w								1	3	1	1	1			7	1.39	0.045	22.14
DDT	control	1	m					1		2		1						4	0.55	0.052	19.40
DDT	control	1	w						4		2							6	1.21	0.052	19.12
DDT	control	2	m					1		1	1							3	0.40	0.052	19.08
DDT	control	2	w							n.E.								0			
DDT	control	3	m							n.E.								0			
DDT	control	3	w							n.E.								0			
DDT	control	4	m				2	4	1									7	0.61	0.058	17.33
DDT	control	4	w						2									2	1.01	0.054	18.50
DDT	control	5	m					2	4		1							7	0.63	0.054	18.46
DDT	control	5	w					2	4		2	1						9	1.55	0.053	18.96
DDT	8.1	1-5								n.E.								0			
DDT	2.7	1	m							n.E.								0			
DDT	2.7	1	w							n.E.								0			
DDT	2.7	2	m							n.E.								0			
DDT	2.7	2	w				1				1							2	1.05	0.055	18.28
DDT	2.7	3	m							n.E.								0			
DDT	2.7	3	w						1			1						2		0.050	19.89
DDT	2.7	4	m							n.E.								0			
DDT	2.7	4	w					1										1	1.25	0.057	17.50
DDT	2.7	5	m							1								1	0.00	0.051	19.50
DDT	2.7	5	w							n.E.								0			
DDT	0.9	1	m									1						1	0.10	0.047	21.50
DDT	0.9	1	w								1		1		1			3	1.17	0.045	22.38
DDT	0.9	2	m				2											2	0.43	0.061	16.50
DDT	0.9	2	w				1	4										5	1.29	0.058	17.29
DDT	0.9	3	m			1	3	2		1								7	0.60	0.059	16.99
DDT	0.9	3	w				1	2	5		1		2	1				12	1.41	0.052	19.19
DDT	0.9	4	m			3	2				1							6	0.57	0.061	16.50
DDT	0.9	4	w				2	3	2	2		1					1	11	1.58	0.053	18.72
DDT	0.9	5	m							n.E.								0			
DDT	0.9	5	w												1			1	1.45	0.041	24.50
DDT	0.3	1	m				7	4										11	0.61	0.059	16.85
DDT	0.3	1	w					1	2	5								8	1.40	0.053	18.97
DDT	0.3	2	m			3	4	1										8	0.65	0.062	16.22
DDT	0.3	2	w				4	3		3								10	1.56	0.057	17.61
DDT	0.3	3	m					1	1									2	0.32	0.056	17.99
DDT	0.3	3	w							2	1							3	1.31	0.050	19.82
DDT	0.3	4	m			2	4	2		2								10	0.58	0.059	17.00
DDT	0.3	4	w				1	3	1	2								7	1.45	0.056	18.01
DDT	0.3	5	m			4	6		1									11	0.61	0.061	16.28
DDT	0.3	5	w				3	4	3									10	1.61	0.057	17.47
DDT	0.1	1	m				4	3	1									8	0.42	0.058	17.10
DDT	0.1	1	w					2	7	1	2							12	1.42	0.053	18.71
DDT	0.1	2	m			1	7	3	1									12	0.60	0.060	16.80
DDT	0.1	2	w				1	2	2	2								7	1.34	0.055	18.16
DDT	0.1	3	m			2	3	1										6	0.57	0.061	16.30
DDT	0.1	3	w			1	4	5	1									11	1.44	0.059	17.01
DDT	0.1	4	m			11	3	1										15	0.73	0.063	15.81
DDT	0.1	4	w				1	3	1									5	1.43	0.057	17.48
DDT	0.1	5	m					1	2	3	1		2					9	0.67	0.051	19.70
DDT	0.1	5	w							1	2	3			1		1	8	1.58	0.046	21.80
PCP	sc	1	m		4	2			2	1		1						10	0.69	0.061	16.37
PCP	sc	1	w					1	1	3		1						6	1.50	0.052	19.26
PCP	sc	2	m	2	6	2												10	0.64	0.069	14.47
PCP	sc	2	w		2	3	4	1										10	1.48	0.063	15.85
PCP	sc	3	m				1	7	3									11	0.67	0.057	17.66
PCP	sc	3	w				2		1	1		3						7	1.45	0.052	19.12
PCP	sc	4	m				3	1	1	2		2						9	0.62	0.054	18.42
PCP	sc	4	w				1		2	2		3			1			9	1.40	0.050	19.93
PCP	sc	5	m	3	10	1												14	0.64	0.070	14.34
PCP	sc	5	w		1	3	2											6	1.17	0.064	15.64
PCP	control	1	m				1	3	1	1								6	0.64	0.056	17.78
PCP	control	1	w					1		1		1						3	1.40	0.052	19.36
PCP	control	2	m	2	6	3												11	0.69	0.069	14.56
PCP	control	2	w		1	5	3											9	1.48	0.064	15.70
PCP	control	3	m	1	7	3												11	0.82	0.068	14.66
PCP	control	3	w		2	5	1	1										9	1.51	0.064	15.56
PCP	control	4	m			1	1	1	1			2						6	0.64	0.055	18.22
PCP	control	4	w				1	2				6		1				10	1.34	0.050	20.14
PCP	control	5	m	3	3	1												7	0.73	0.071	14.18
PCP	control	5	w			9	2	1	1									13	1.39	0.063	15.99
PCP	500	1-5								n.E.								0			
PCP	50	1	m		4	5	2		1									12	0.54	0.064	15.51
PCP	50	1	w				2	3	1	2								8	1.21	0.056	17.81

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	conc.			number of emerging midges at day														i. dw	dr	EMT_{50}
chemical	[mg kg^{-1}]	rep.	sex	14	15	16	17	18	19	20	21	22	23	24	25	26	27	E	[mg]	[d^{-1}]	[d]
PCP	50	2	m					1		2		2		1				6	0.49	0.049	20.32
PCP	50	2	w									3		1	1			5	1.10	0.045	22.43
PCP	50	3	m				2	5										7	0.50	0.058	17.20
PCP	50	3	w					8	3									11	1.14	0.056	17.76
PCP	50	4	m				10	1										11	0.48	0.060	16.59
PCP	50	4	w					2	5	1								8	1.17	0.054	18.36
PCP	50	5	m			2	3									1		6	0.48	0.058	17.14
PCP	50	5	w					4	6	1								11	1.14	0.055	18.21
PCP	5	1	m	1	6	1	2											10	0.59	0.067	14.85
PCP	5	1	w			4	4	2	1									11	-	0.061	16.45
PCP	5	2	m	1	6	2	1		1									11	0.61	0.067	15.04
PCP	5	2	w		1	2	3	2										8	1.22	0.062	16.19
PCP	5	3	m		8	1	1											10	0.59	0.068	14.77
PCP	5	3	w		1	2	3		1									7	1.35	0.062	16.13
PCP	5	4	m	1	6	2												9	0.64	0.069	14.59
PCP	5	4	w			5	6	1										12	1.38	0.062	16.14
PCP	5	5	m		1		2	2										5	0.55	0.061	16.42
PCP	5	5	w											1				1	1.04	0.043	23.50
PCP	0.5	1	m		5	2	1											8	0.63	0.067	14.97
PCP	0.5	1	w			1	5	3	1									10	1.30	0.059	16.86
PCP	0.5	2	m		6	5												11	0.65	0.067	14.94
PCP	0.5	2	w			1	4	3										8	1.38	0.060	16.72
PCP	0.5	3	m		9	2	1											12	0.58	0.068	14.81
PCP	0.5	3	w			3	2	2	1									8	1.36	0.060	16.56
PCP	0.5	4	m												1			1	0.59	0.041	24.50
PCP	0.5	4	w							n.E.								0			
PCP	0.5	5	m	1	5	1												7	0.65	0.069	14.48
PCP	0.5	5	w		2	4	4		1			1		1				13	1.36	0.060	16.63
PCP	0.05	1	m		7													7	0.61	0.069	14.50
PCP	0.05	1	w			4	4	1	2									11	1.39	0.061	16.52
PCP	0.05	2	m					1	2									3	0.69	0.055	18.15
PCP	0.05	2	w					1	3			2						6	1.31	0.052	19.21
PCP	0.05	3	m	2	2	5	1											10	0.72	0.067	14.94
PCP	0.05	3	w			3	1	4		1		1						10	1.53	0.058	17.23
PCP	0.05	4	m		1	5	1		1			1			1			10	0.62	0.059	16.85
PCP	0.05	4	w			2		2	2			1						7	1.32	0.057	17.59
PCP	0.05	5	m			4	1	1	1	1								8	0.69	0.060	16.63
PCP	0.05	5	w				1	2	3	3		2			1			12	1.47	0.052	19.21
TBT-Cl	sc	1	m				1	2	1		1							5	0.70	0.056	18.00
TBT-Cl	sc	1	w							2	1							3	1.51	0.050	19.82
TBT-Cl	sc	2	m				1	2	2		1							6	0.70	0.055	18.08
TBT-Cl	sc	2	w						1									1	1.63	0.054	18.50
TBT-Cl	sc	3	m		3	1	1		1									6	0.70	0.064	15.54
TBT-Cl	sc	3	w			1	5	5	1	1								13	1.51	0.058	17.14
TBT-Cl	sc	4	m					1			2							3	0.79	0.052	19.39
TBT-Cl	sc	4	w							2		1						3	1.53	0.050	20.12
TBT-Cl	sc	5	m				1	4	2	2	2	2						13	0.70	0.053	18.83
TBT-Cl	sc	5	w					1	1	2		1	1					6	1.52	0.051	19.69
TBT-Cl	control	1	m			6	5											11	0.72	0.063	15.94
TBT-Cl	control	1	w				2	6	1									9	1.58	0.058	17.37
TBT-Cl	control	2	m		6	1		1										8	0.88	0.067	14.94
TBT-Cl	control	2	w		2	7	2		1									12	1.51	0.064	15.69
TBT-Cl	control	3	m		3	9												12	0.63	0.066	15.24
TBT-Cl	control	3	w			4	3	1										8	1.44	0.062	16.10
TBT-Cl	control	4	m				4	2		1	1							8	0.85	0.057	17.51
TBT-Cl	control	4	w					1		1	2		1					5	1.45	0.050	19.97
TBT-Cl	control	5	m		1	6	1											8	0.66	0.065	15.48
TBT-Cl	control	5	w				4	5		1								10	1.37	0.058	17.26
TBT-Cl	16	1-5								n.E.								0			
TBT-Cl	8	1	m							2		2	1					5	0.56	0.048	20.83
TBT-Cl	8	1	w							1		1	2			2		6	0.92	0.044	22.63
TBT-Cl	8	2	m							n.E.								0			
TBT-Cl	8	2	w												1			1	1.68	0.041	24.50
TBT-Cl	8	3	m				3	4	1		1							9	0.58	0.057	17.54
TBT-Cl	8	3	w					2	2	1	1				1			7	0.89	0.052	19.27
TBT-Cl	8	4	m									3				1		4	0.56	0.045	22.38
TBT-Cl	8	4	w									1				2		3	0.89	0.042	24.01
TBT-Cl	4	1	m		1	4	1	1										7	0.67	0.064	15.74
TBT-Cl	4	1	w					2	4	1								7	1.43	0.055	18.33
TBT-Cl	4	2	m						1									1	1.12	0.054	18.50
TBT-Cl	4	2	w							n.E.								0			
TBT-Cl	4	3	m			3	5	3										11	0.64	0.061	16.47
TBT-Cl	4	3	w					2	1	1	1							5	1.49	0.054	18.63
TBT-Cl	4	4	m				2	5	3									10	0.71	0.057	17.57
TBT-Cl	4	4	w						3	2	2	1						8	1.65	0.051	19.57
TBT-Cl	4	5	m					1										1	0.49	0.057	17.50
TBT-Cl	4	5	w							n.E.								0			
TBT-Cl	2	1	m				2	6										8	0.80	0.058	17.24
TBT-Cl	2	1	w					1	5	3								9	1.57	0.053	18.70
TBT-Cl	2	2	m					1	2	3	2					1		9	0.75	0.051	19.74
TBT-Cl	2	2	w						2	1	2	1	1	1				8	1.59	0.049	20.49
TBT-Cl	2	3	m				3	2	2	2	1							10	0.67	0.056	18.00
TBT-Cl	2	3	w					1	1	1	1	3		1				8	1.51	0.049	20.34
TBT-Cl	2	4	m		2	4	2		1									9	0.66	0.063	15.75
TBT-Cl	2	4	w			1	6	3		1								11	1.39	0.059	16.90
TBT-Cl	2	5	m					2	3	1	1	1						8	0.74	0.053	18.91
TBT-Cl	2	5	w							2	4	2	1					9	1.60	0.048	20.68
TBT-Cl	1	1	m			5	5	1			1							12	0.71	0.061	16.40
TBT-Cl	1	1	w				3	2	4									9	1.55	0.057	17.57
TBT-Cl	1	2	m				3	2		2		1						8	0.66	0.056	17.97

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	conc.			number of emerging midges at day														i. dw	dr	EMT_{50}
chemical	[mg kg^{-1}]	rep.	sex	14	15	16	17	18	19	20	21	22	23	24	25	26	27	E	[mg]	[d^{-1}]	[d]
TBT-Cl	1	2	w					1	3	1	1	2						8	1.46	0.052	19.40
TBT-Cl	1	3	m			1		3	1		1							6	0.67	0.056	17.71
TBT-Cl	1	3	w			1		2	1	2	2	2						10	1.51	0.053	19.01
TBT-Cl	1	4	m				1	6	1	1	1							10	0.89	0.056	17.93
TBT-Cl	1	4	w						1	2		1						4	1.26	0.051	19.69
TBT-Cl	1	5	m					1	2	4	1							8	0.51	0.052	19.09
TBT-Cl	1	5	w									5	5	1	1			12	1.10	0.045	22.30
TNT	sc	1	m		5	2					1							8	0.63	0.065	15.31
TNT	sc	1	w			4	7					1						12	1.42	0.061	16.47
TNT	sc	2	m			1	2	3		3	1	1						11	0.67	0.055	18.14
TNT	sc	2	w						1	2	1							4	1.47	0.051	19.47
TNT	sc	3	m					4	1									5	0.69	0.057	17.69
TNT	sc	3	w					5	6	2								13	1.51	0.055	18.24
TNT	sc	4	m			2	2		1									5	0.62	0.061	16.43
TNT	sc	4	w			1	5	7	1									14	1.39	0.059	17.04
TNT	sc	5	m			5	2											7	0.73	0.063	15.77
TNT	sc	5	w				3	4	2	2								11	1.57	0.056	17.71
TNT	control	1	m		2	7	1											10	0.68	0.065	15.38
TNT	control	1	w				4	3	3									10	1.45	0.058	17.36
TNT	control	2	m		1	7	1		1									10	0.66	0.064	15.74
TNT	control	2	w				5	5				1						11	1.55	0.058	17.32
TNT	control	3	m		2	5	2	1	1									11	0.66	0.063	15.87
TNT	control	3	w				6	1										7	1.50	0.060	16.64
TNT	control	4	m		1	6	4											11	0.68	0.063	15.75
TNT	control	4	w				1	8										9	1.53	0.058	17.38
TNT	control	5	m				1	2	1	1								5	0.73	0.056	17.84
TNT	control	5	w						3			3	1					7	1.47	0.049	20.22
TNT	200	1	m											1			1	2	0.63	0.040	24.91
TNT	200	1	w											1	1	2	1	5	1.30	0.040	25.06
TNT	200	2	m								3	1	1					5	0.89	0.047	21.07
TNT	200	2	w							2		1	1	1				5	1.58	0.047	21.18
TNT	200	3	m					1	6	2								9	0.75	0.054	18.59
TNT	200	3	w							5	3	2						10	1.55	0.050	20.17
TNT	200	4	m									1						1	0.66	0.047	21.50
TNT	200	4	w											1				1	1.35	0.043	23.50
TNT	200	5	m					9	1	1								11	0.71	0.056	17.75
TNT	200	5	w						3	3			2		1			9	1.52	0.050	20.19
TNT	80	1	m				2	3					1					6	0.75	0.056	17.80
TNT	80	1	w					3	3		3	1	2			1		13	1.47	0.050	19.86
TNT	80	2	m				5	4	3									12	0.73	0.058	17.30
TNT	80	2	w					1	4		1							6	1.58	0.054	18.63
TNT	80	3	m			4	4	2										10	0.61	0.061	16.27
TNT	80	3	w				4	3						1				8	1.38	0.057	17.53
TNT	80	4	m			4	6	4										14	0.75	0.061	16.47
TNT	80	4	w					3		3								6	1.62	0.054	18.45
TNT	80	5	m						2	4				1				7	0.69	0.051	19.67
TNT	80	5	w							1	5		2	1	2	1		12	1.32	0.046	21.92
TNT	32	1	m			2	8	1										11	0.66	0.061	16.39
TNT	32	1	w				1	3	3			1						8	1.49	0.055	18.15
TNT	32	2	m		1	7	1											9	0.66	0.065	15.49
TNT	32	2	w				8	1	2									11	1.37	0.059	16.92
TNT	32	3	m				2	6	1	1								10	0.75	0.057	17.56
TNT	32	3	w					3	5	1								9	1.62	0.055	18.26
TNT	32	4	m			1	3	1	1									6	0.68	0.060	16.78
TNT	32	4	w				1	6	2	3	1		1					14	1.46	0.054	18.45
TNT	32	5	m									7		2		1		10	0.74	0.045	22.23
TNT	32	5	w									2	2	3				7	1.41	0.044	22.61
TNT	12.8	1	m					4	2	1								7	0.63	0.055	18.04
TNT	12.8	1	w						1			1	2			2		6	1.50	0.045	22.40
TNT	12.8	2	m					1				2				2		5	0.72	0.046	21.87
TNT	12.8	2	w									2			2		1	5	1.37	0.042	23.54
TNT	12.8	3	m		7	5	2							1				15	0.68	0.065	15.48
TNT	12.8	3	w				1	4										5	1.42	0.058	17.29
TNT	12.8	4	m			2	5											7	0.73	0.062	16.20
TNT	12.8	4	w				1	8	2	1								12	1.51	0.056	17.72
TNT	12.8	5	m			5	4	1										10	0.72	0.062	16.07
TNT	12.8	5	w				2	5	2									9	1.50	0.057	17.47
TNT	5.12	1	m						2	2	2		1					7	0.67	0.050	19.85
TNT	5.12	1	w											2				2	1.44	0.043	23.50
TNT	5.12	2	m				1	3	2	4	2		1					13	0.73	0.053	18.92
TNT	5.12	2	w						1	3								4	1.54	0.052	19.24
TNT	5.12	3	m			3	8	1										12	0.66	0.061	16.31
TNT	5.12	3	w				1	3	3	1								8	1.49	0.056	17.96
TNT	5.12	4	m			4	1	2		1								8	0.68	0.061	16.52
TNT	5.12	4	w			1	5	4		1	1		1					13	1.54	0.057	17.55
TNT	5.12	5	m		3	4												7	0.64	0.066	15.06
TNT	5.12	5	w			1	6	3		1					1			12	1.47	0.058	17.35

m = male, f = female, n.E. = no emergence, sc = solvent control